

Supplementary Information for

**Single-cell spatial metabolomics with cell-type specific protein profiling
for tissue systems biology**

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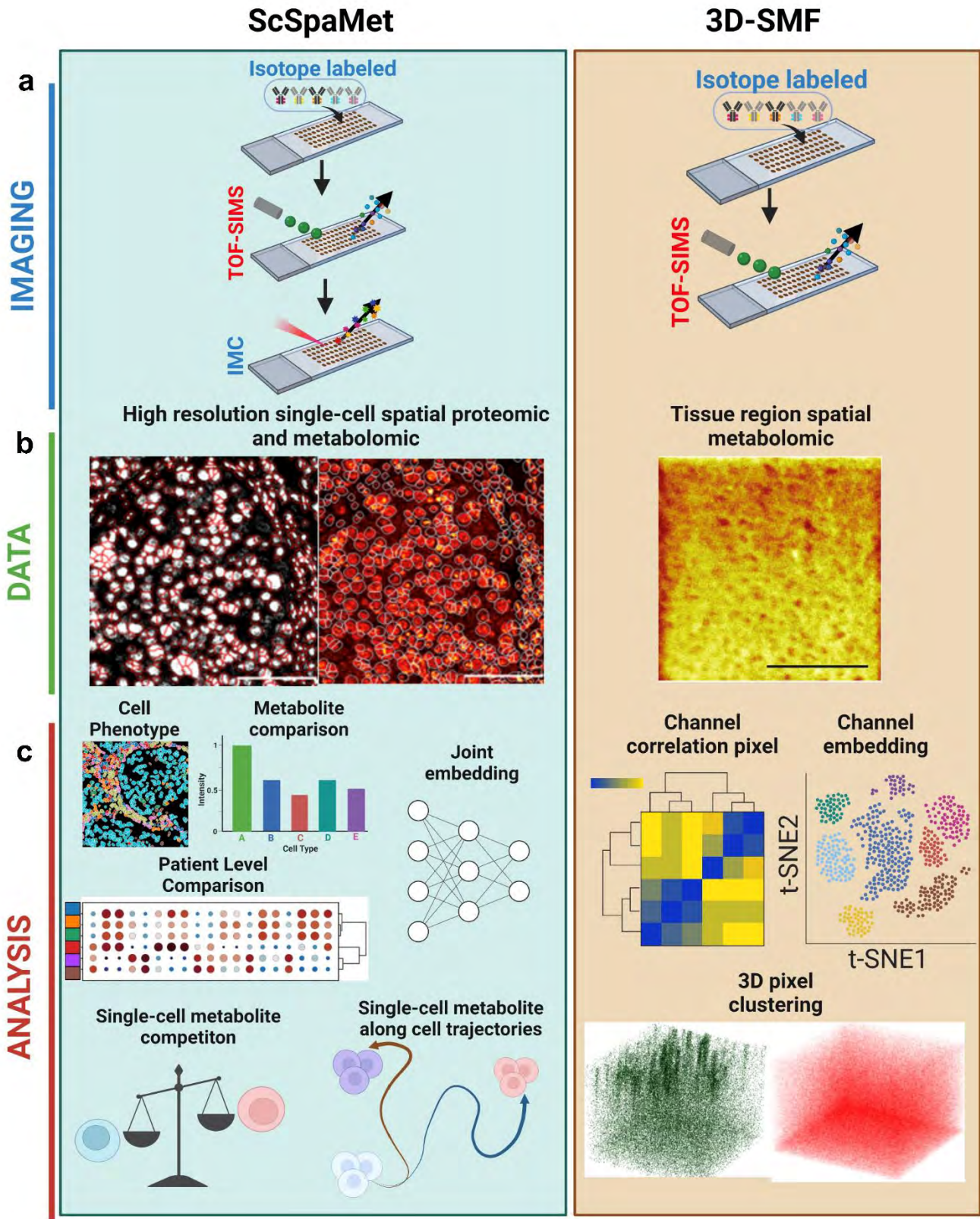
⁷ Interdisciplinary Bioengineering Graduate Program, Georgia Institute of Technology, Atlanta, GA, USA

⁸ Winship Cancer Institute, Emory University, GA, USA

⁹ Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

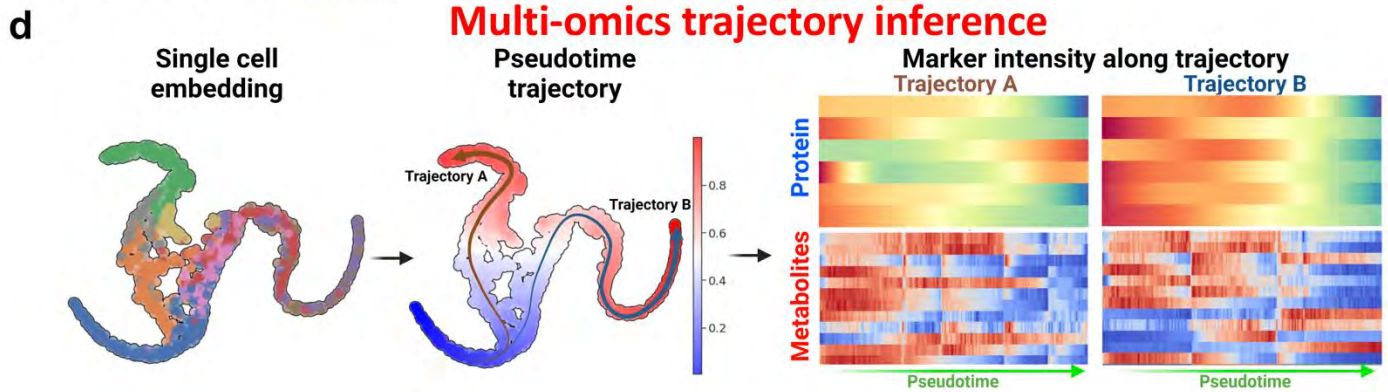
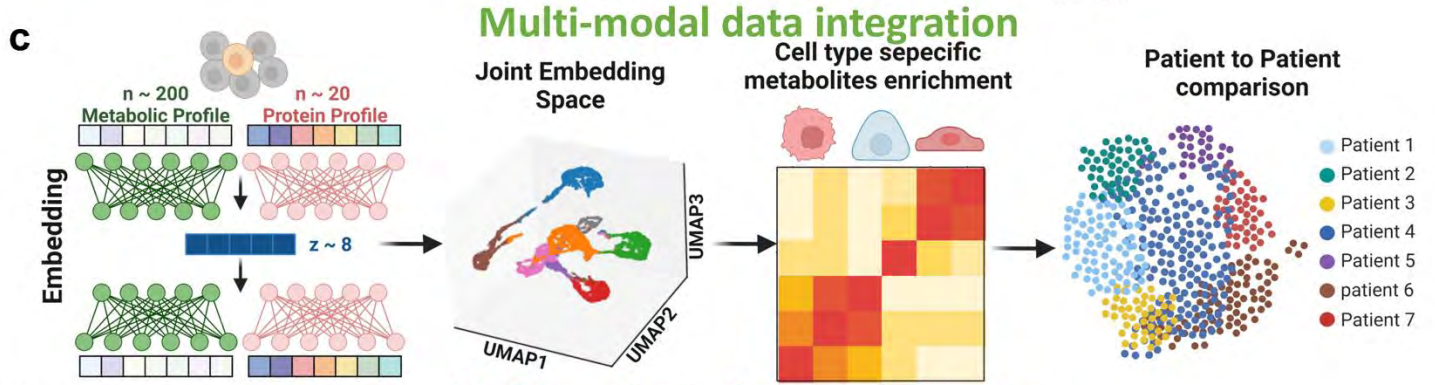
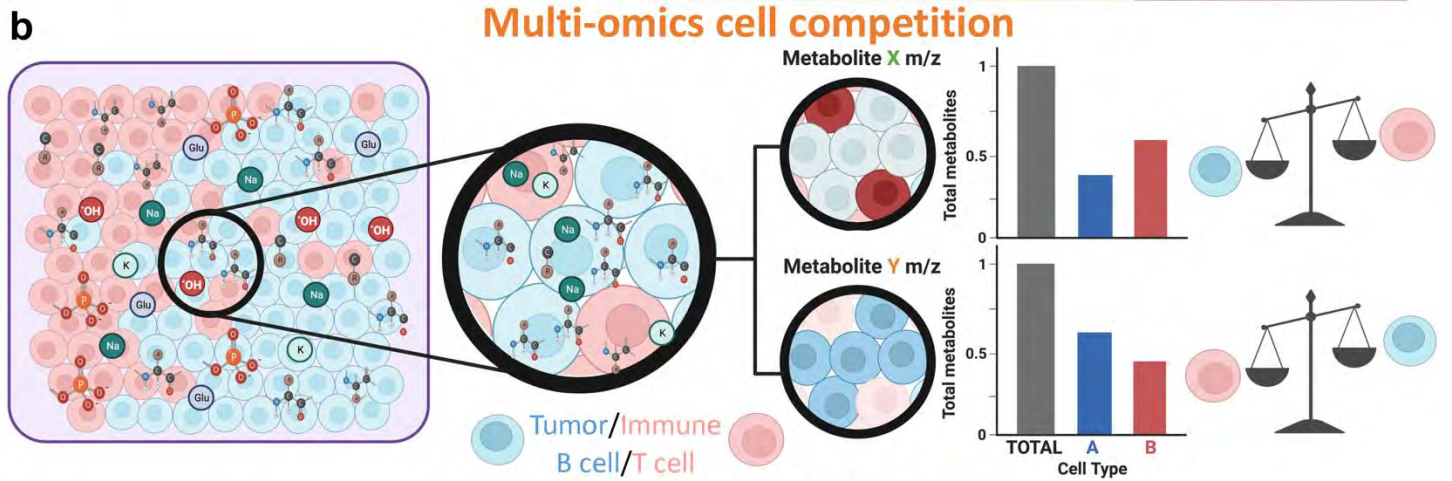
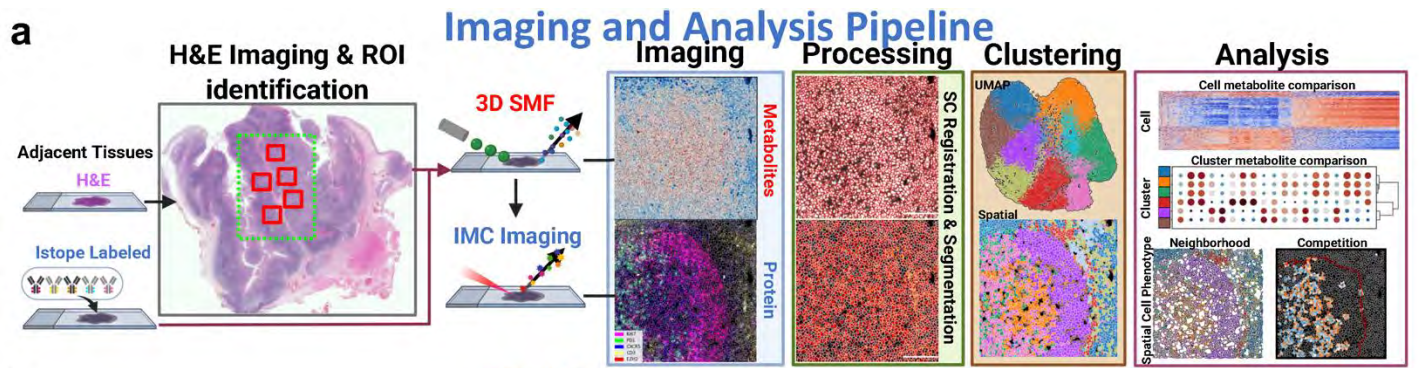
[#]Equal authorship *Corresponding author

Ahmet F. Coskun, Ph.D. (ahmet.coskun@bme.gatech.edu)



Supplementary Fig. 1. Comparison of scSpaMet and 3D-SMF imaging and analysis pipeline.

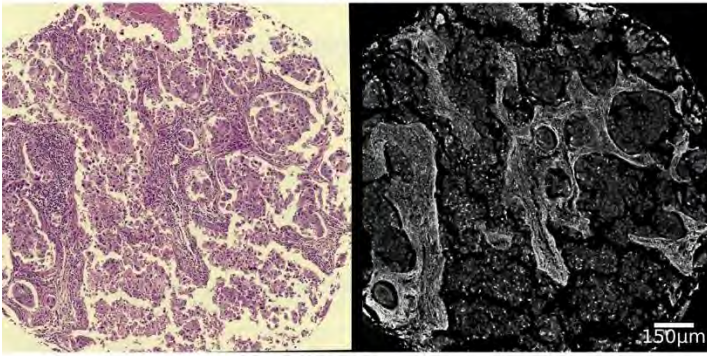
- a** Comparison of scSpaMet and 3D-SMF imaging pipeline. ScSpaMet advanced the 3D-SMF imaging pipeline by introducing sequential TOF-SIMS and IMC imaging achieving high-resolution single-cell spatial proteomic and metabolomic. Created with Biorender.com
- b** Comparison of scSpaMet and 3D-SMF data output. ScSpaMet achieved high-resolution single-cell imaging and cell segmentation for joint proteomic and metabolomic downstream analysis. 3D-SMF revealed tissue region-specific metabolomic info.
- c** Comparison of scSpaMet and 3D-SMF data analysis. ScSpaMet analyzed single-cell phenotype from proteomic data and compared patient and cell type metabolite variation. ScSpaMet introduced multi-omics cell competition, multi-modal data integration, and multi-omics trajectory inference. The 3D-SMF only analyzed metabolite channel correlation from pixel data, channel embedding, and 3D pixel clustering due to a lack of single-cell information. Created with Biorender.com



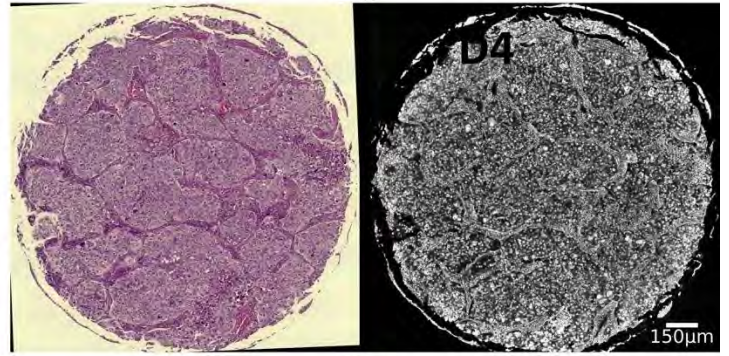
Supplementary Fig. 2. ScSpaMet analysis modules.

- a** Overview of scSpaMet data analysis pipeline. Tissue samples on glass slides are labeled with metal-isotope conjugated antibodies and adjacent tissues stained with H&E are used for the identification of imaging regions for scSpaMet. This is followed by metabolic profiling with 3D-SMF and proteomic profiling using IMC. After imaging, single-cell registration is performed with downstream analysis. Created with Biorender.com
- b** Overview of ScSpaMet local metabolomic competition analysis pipeline. Single cells compete with their neighboring cell for metabolomic resources. ScSpaMet quantifies neighboring cell types' local metabolite competition. Created with Biorender.com
- c** Overview of ScSpaMet single cell joint modality VAE embedding pipeline. Single-cell metabolomic and proteomic profiles are used as input for the VAE to extract joint embedding space representation. Joint embedding is used to decipher cell type metabolic states and enrichment as well as stratify patients. Created with Biorender.com
- d** Overview of ScSpaMet pseudo time analysis pipeline. Using single-cell embedding, cell trajectory differentiation pseudotime analysis is used to measure metabolomic and proteomic change along trajectories. Created with Biorender.com

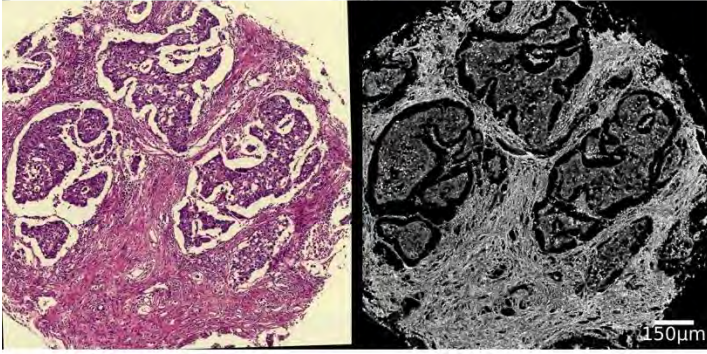
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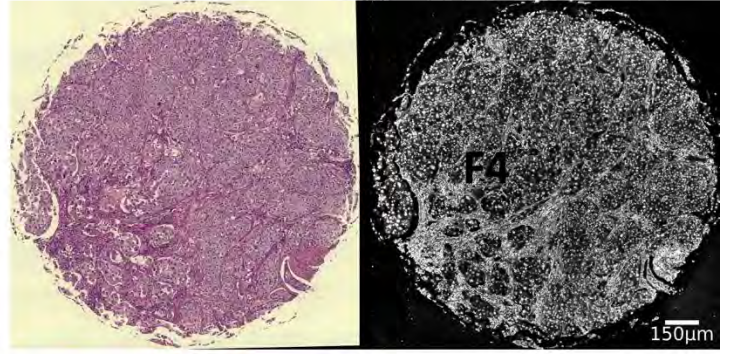
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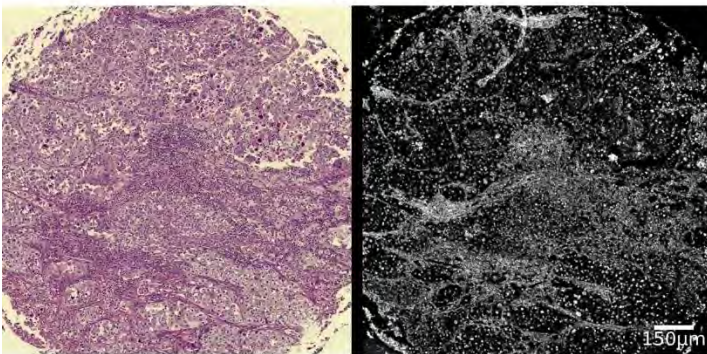
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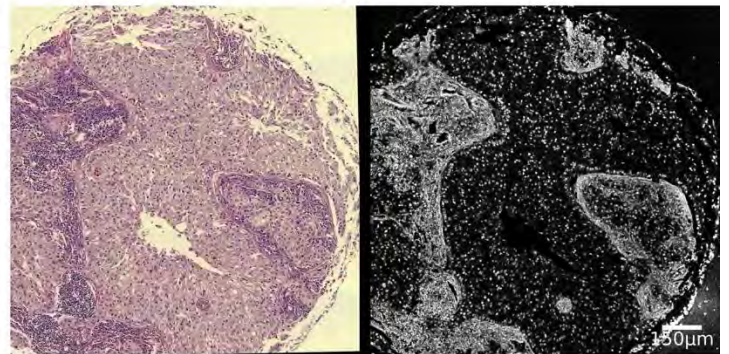
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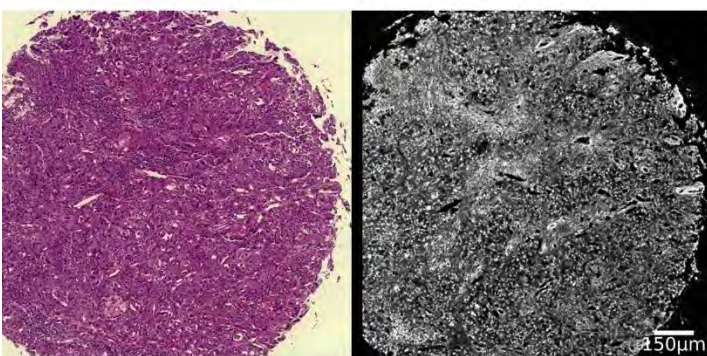
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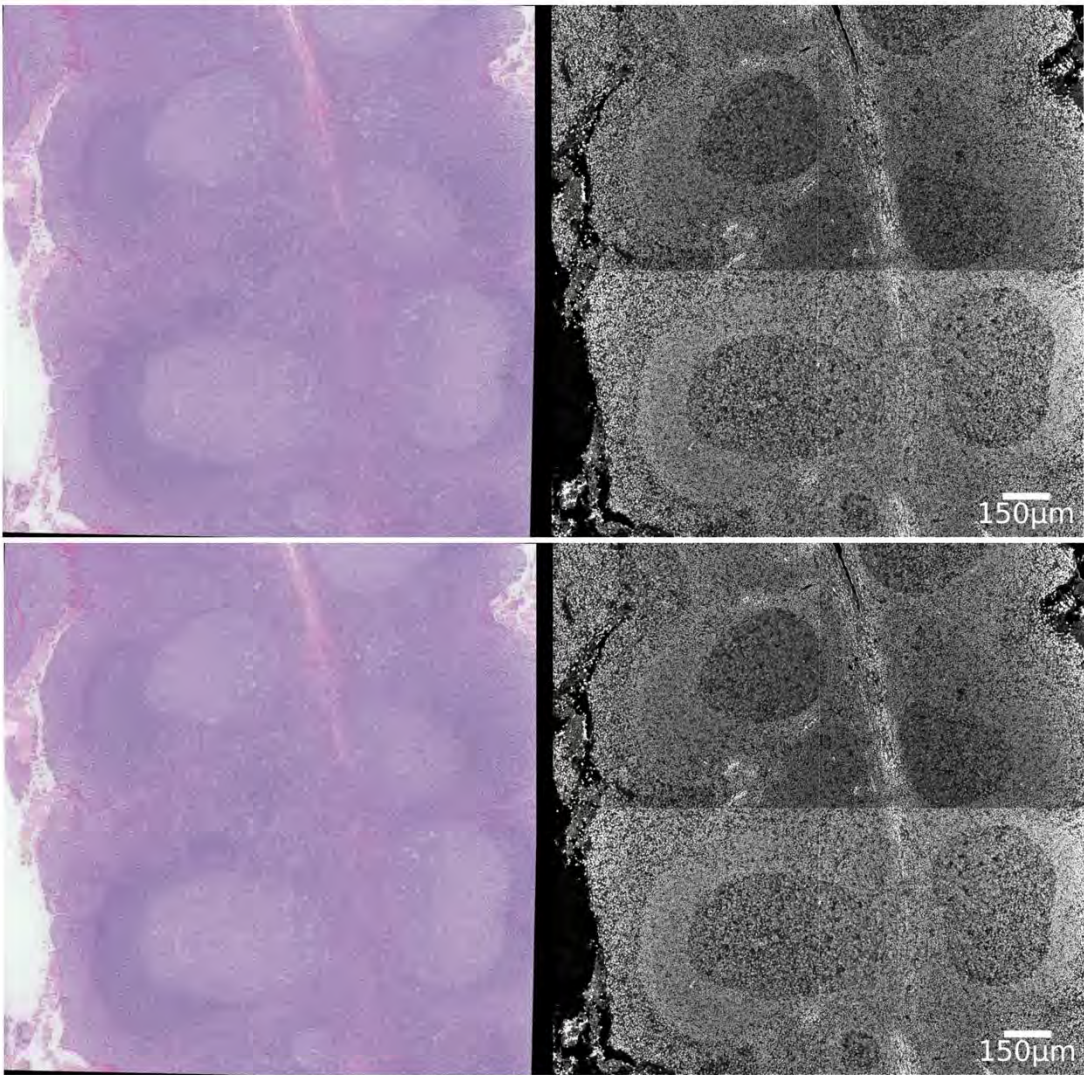
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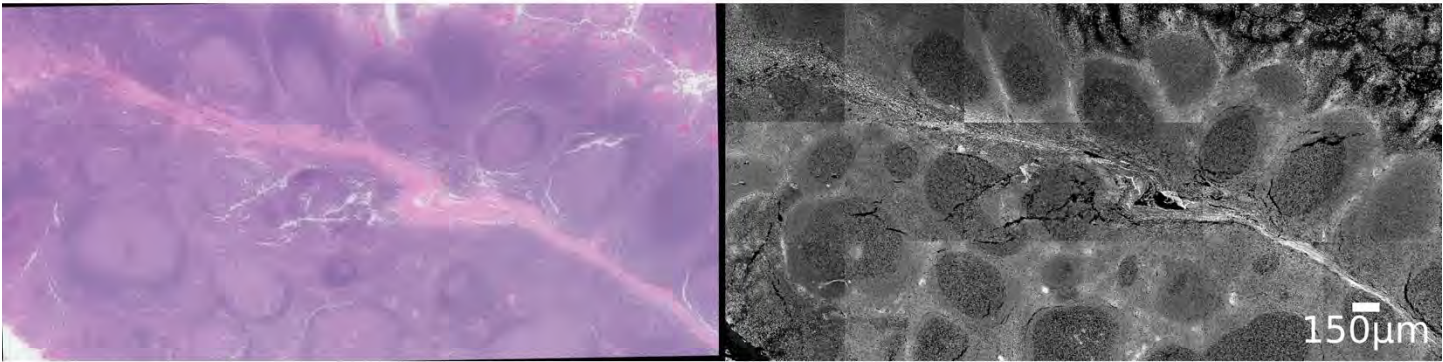
53 **Supplementary Fig. 3.** Matched H&E and IMC Histone H3 marker images in human lung cancer tissues. For
54 each imaged region, the registered H&E stained sequential tissues (left) are shown with the IMC Histone H3
55 marker images (right). Scale bar 150 μm .

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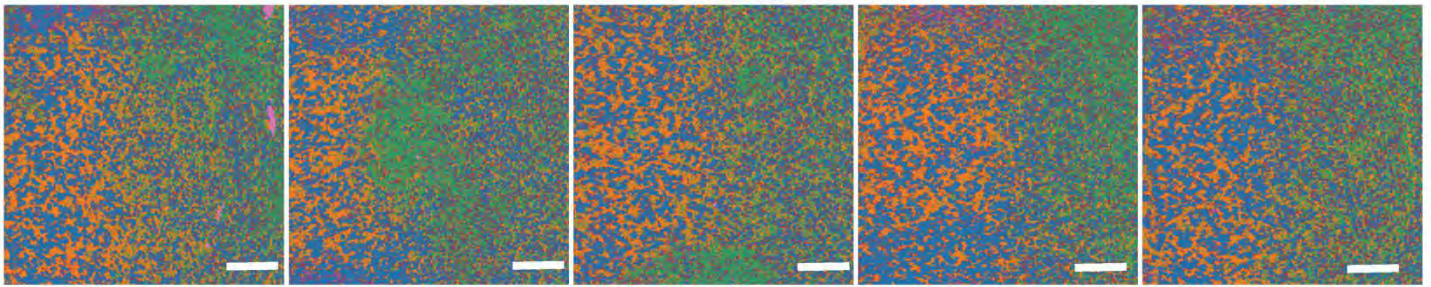


59 **Supplementary Fig. 4.** Matched H&E and IMC Intercalator marker images in human tonsil tissues. For each
60 imaged region, the registered H&E stained sequential tissues (left) are shown with the IMC Histone H3 marker
61 images (right). Scale bar 150 μm .

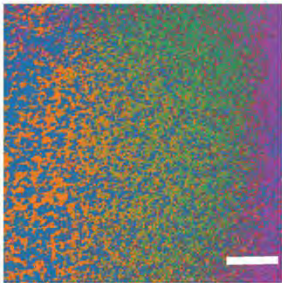
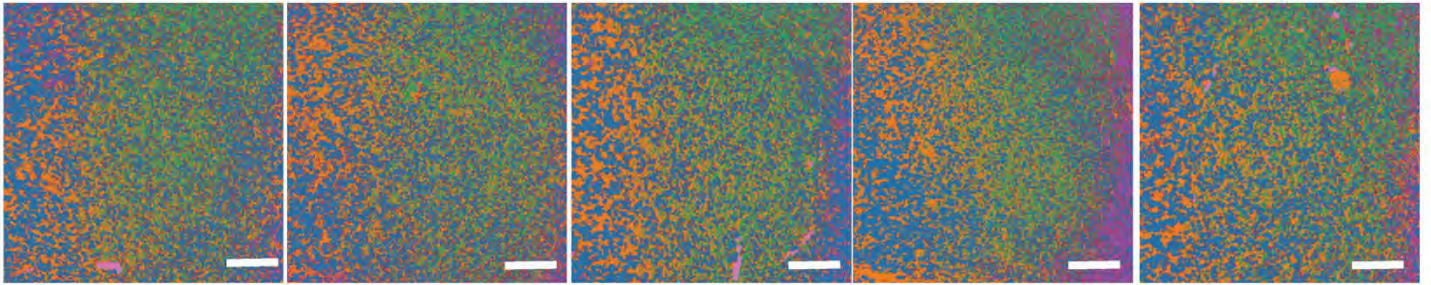
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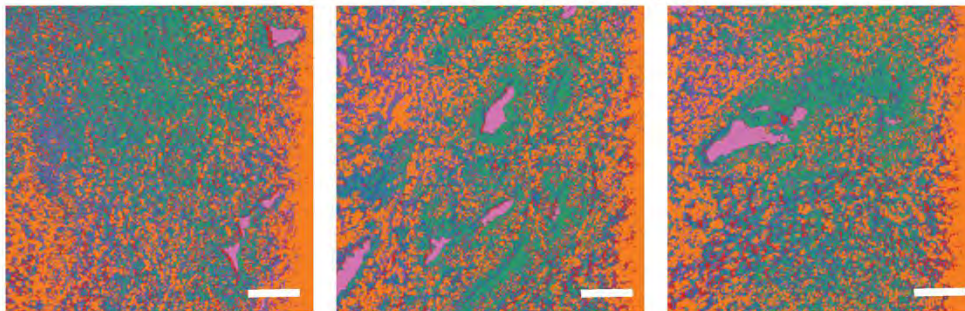
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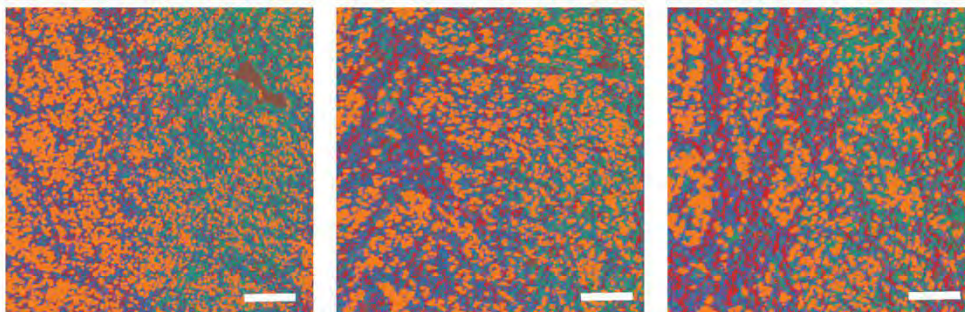
ENDOMETRIUM

b

Obese

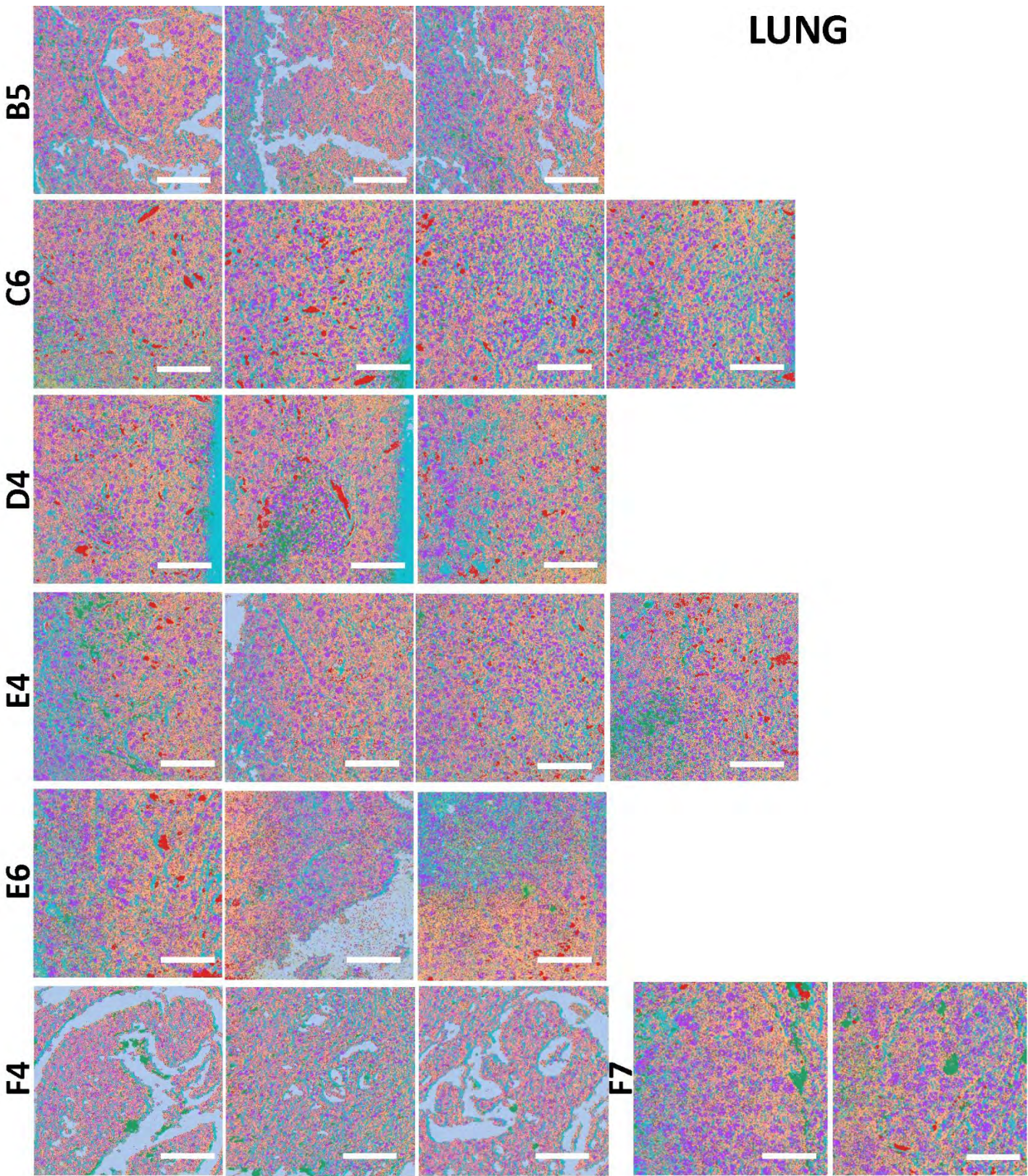


Lean



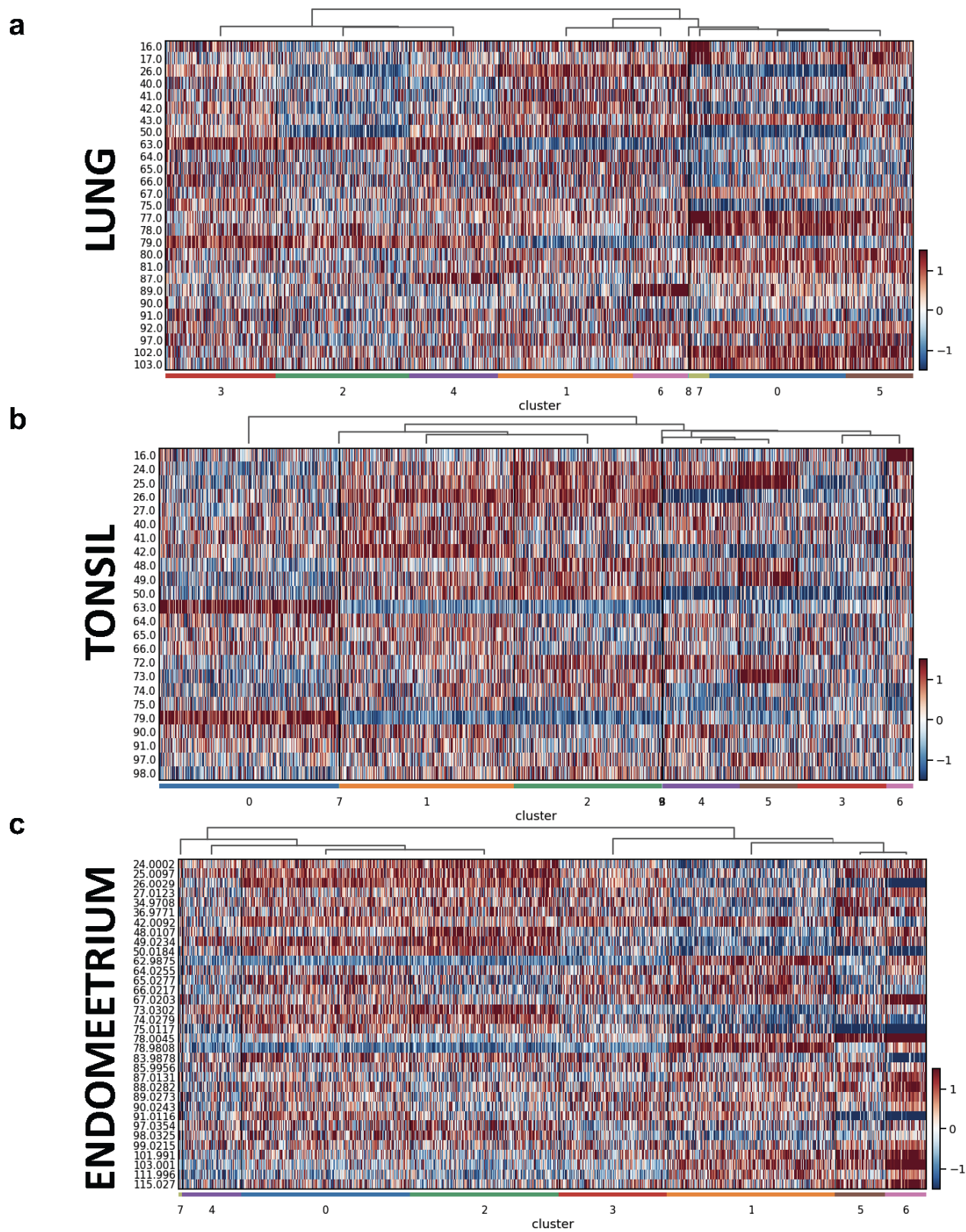
65 **Supplementary Fig. 5.** Pixel clustering for SIMS data in tonsil tissues **a** and endometrium tissues **b**. Scale bar
66 100 μm .

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71 **Supplementary Fig. 6.** Pixel clustering for SIMS data in lung cancer tissues. Scale bar 100 μm .

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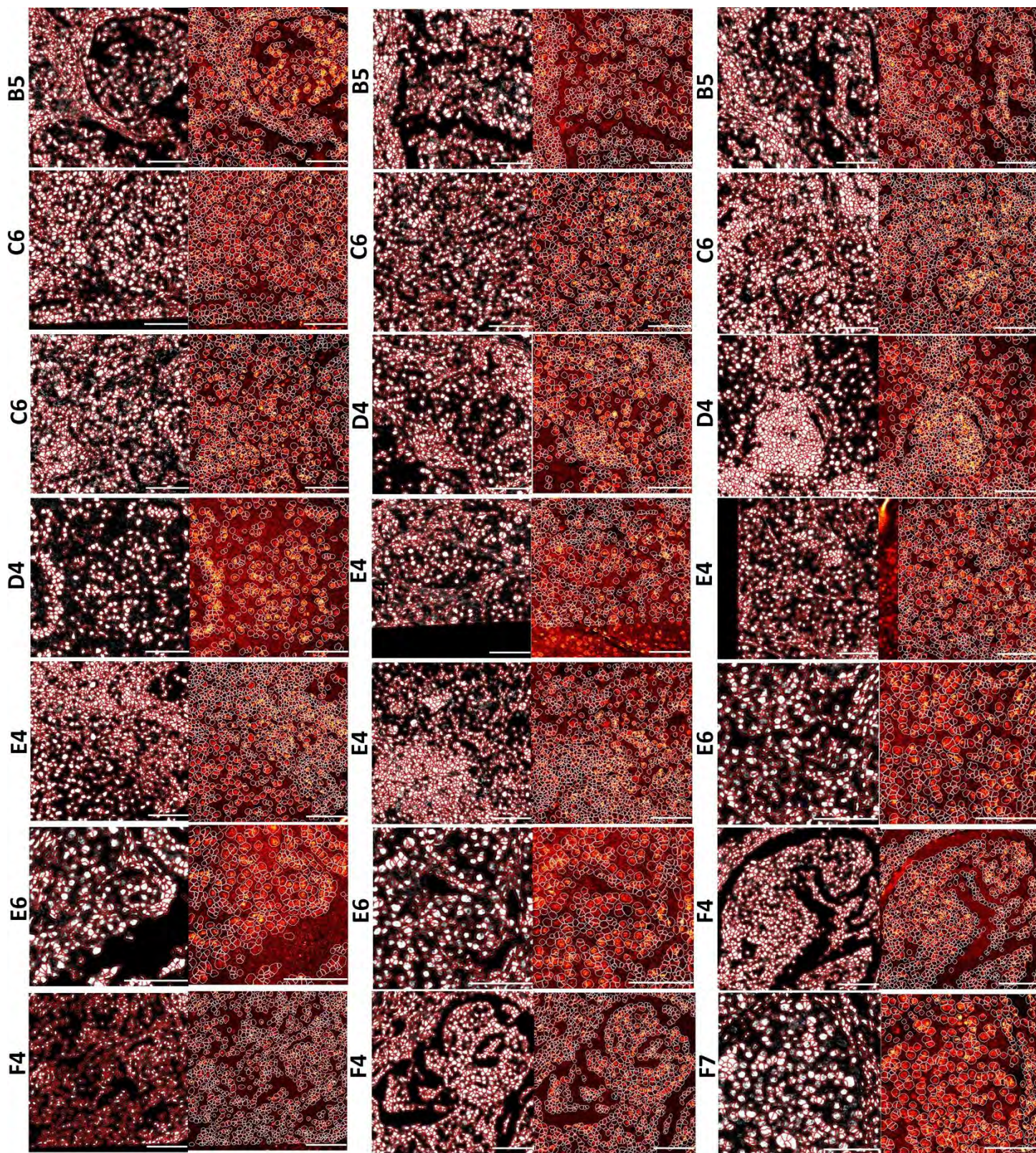


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76 **Supplementary Fig. 7.** Pixel cluster expression for SIMS data for lung **(a)**, tonsil **(b)**, and endometrium **(c)**.
77 Heatmap showing the pixel expression level for selected mass channels. Colormap corresponds to Supplementary
78 Fig. 3-4.

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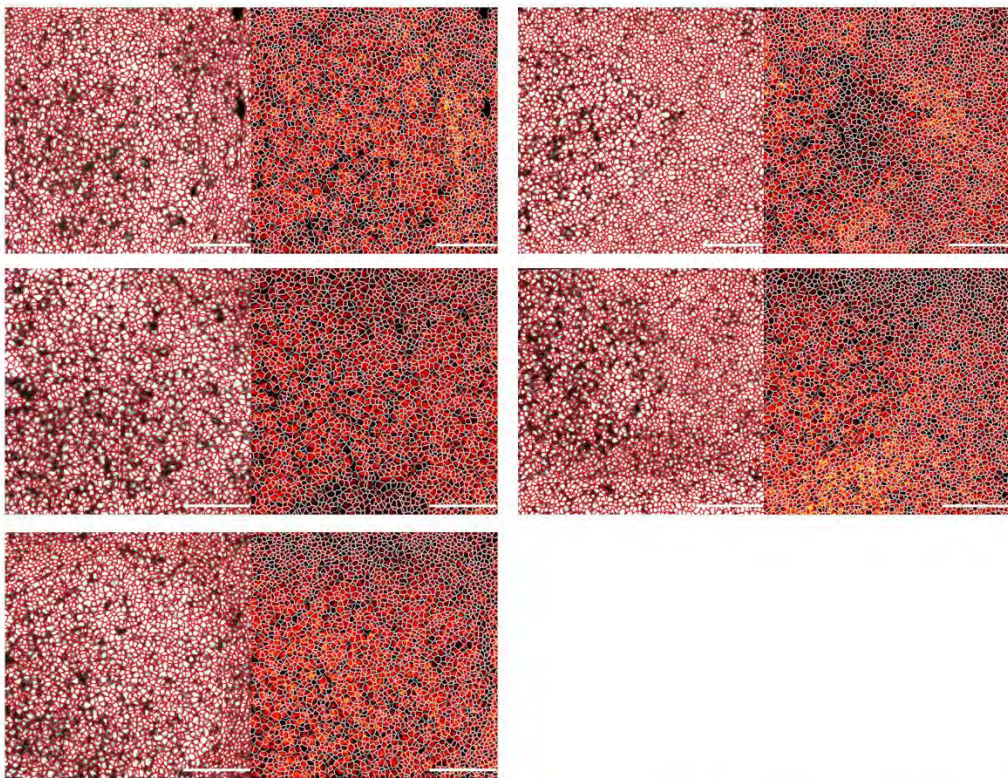
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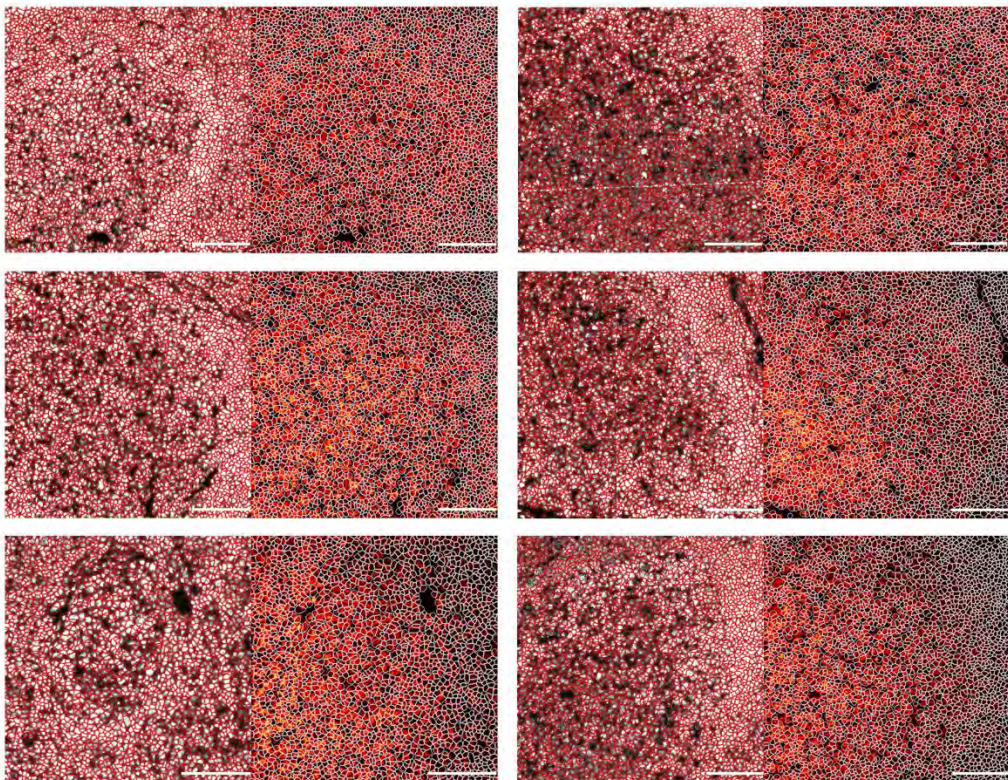
82 **Supplementary Fig. 8.** Single-cell registration results between IMC Histone H3 marker (left) and SIMS PO3-
83 channel (right) in human lung cancer tissues. Scale bar 100 μm .

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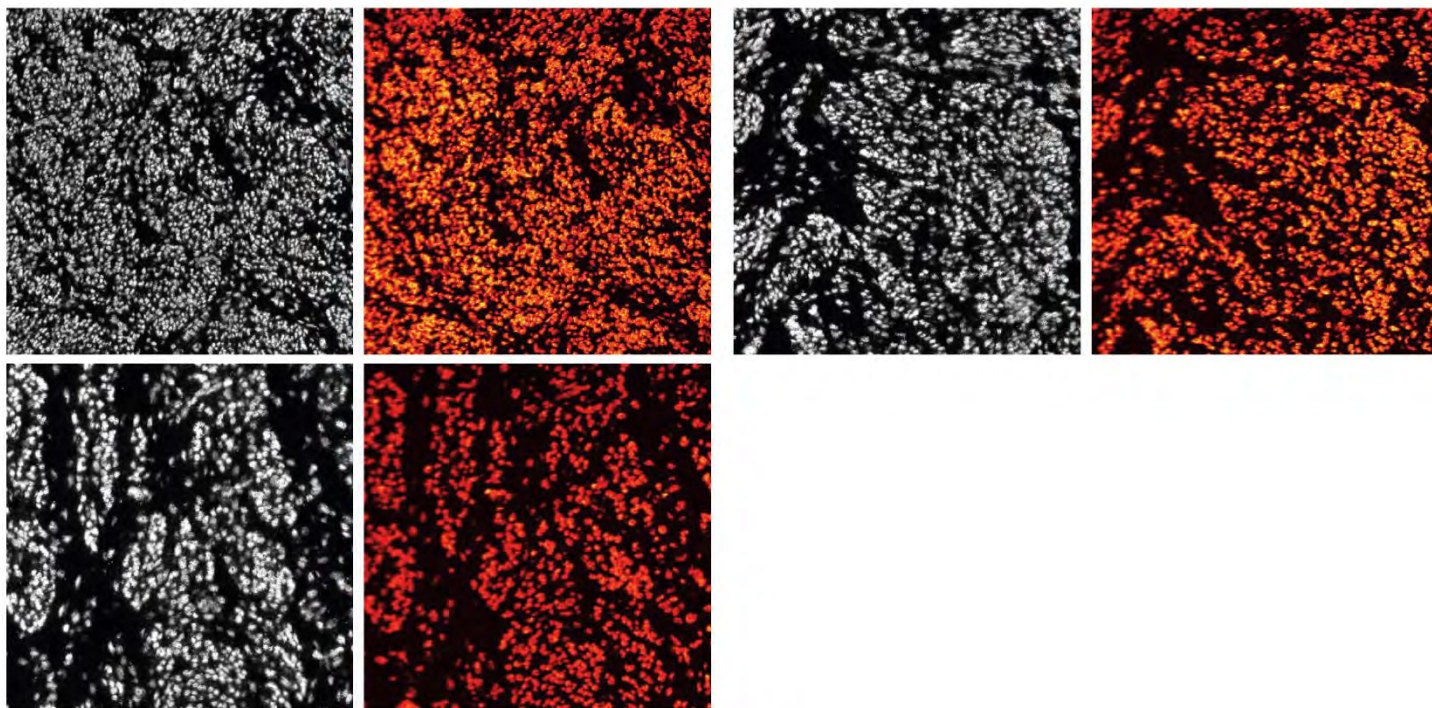
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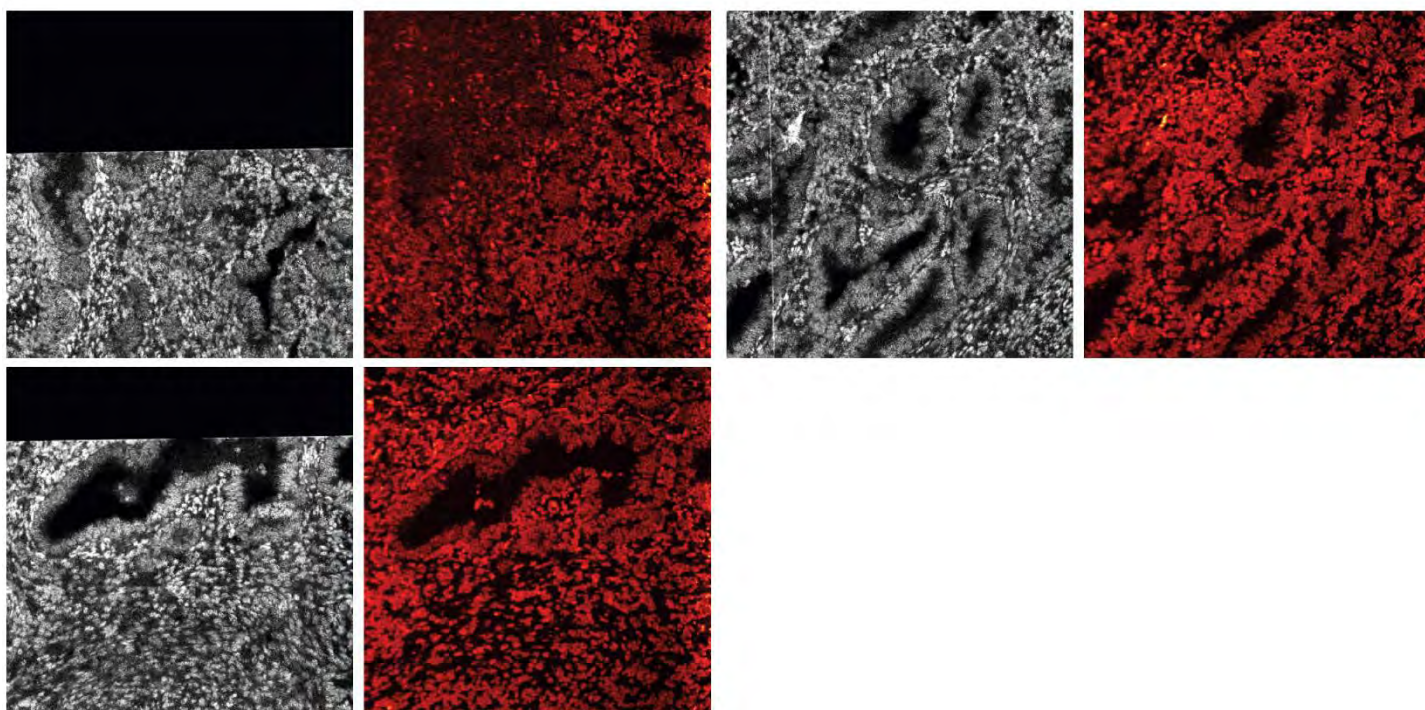
87 **Supplementary Fig. 9.** Single-cell registration results between IMC Intercalator marker (left) and SIMS PO3-
88 channel (right) in human tonsil tissues. Scale bar 100 μm .

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Lean



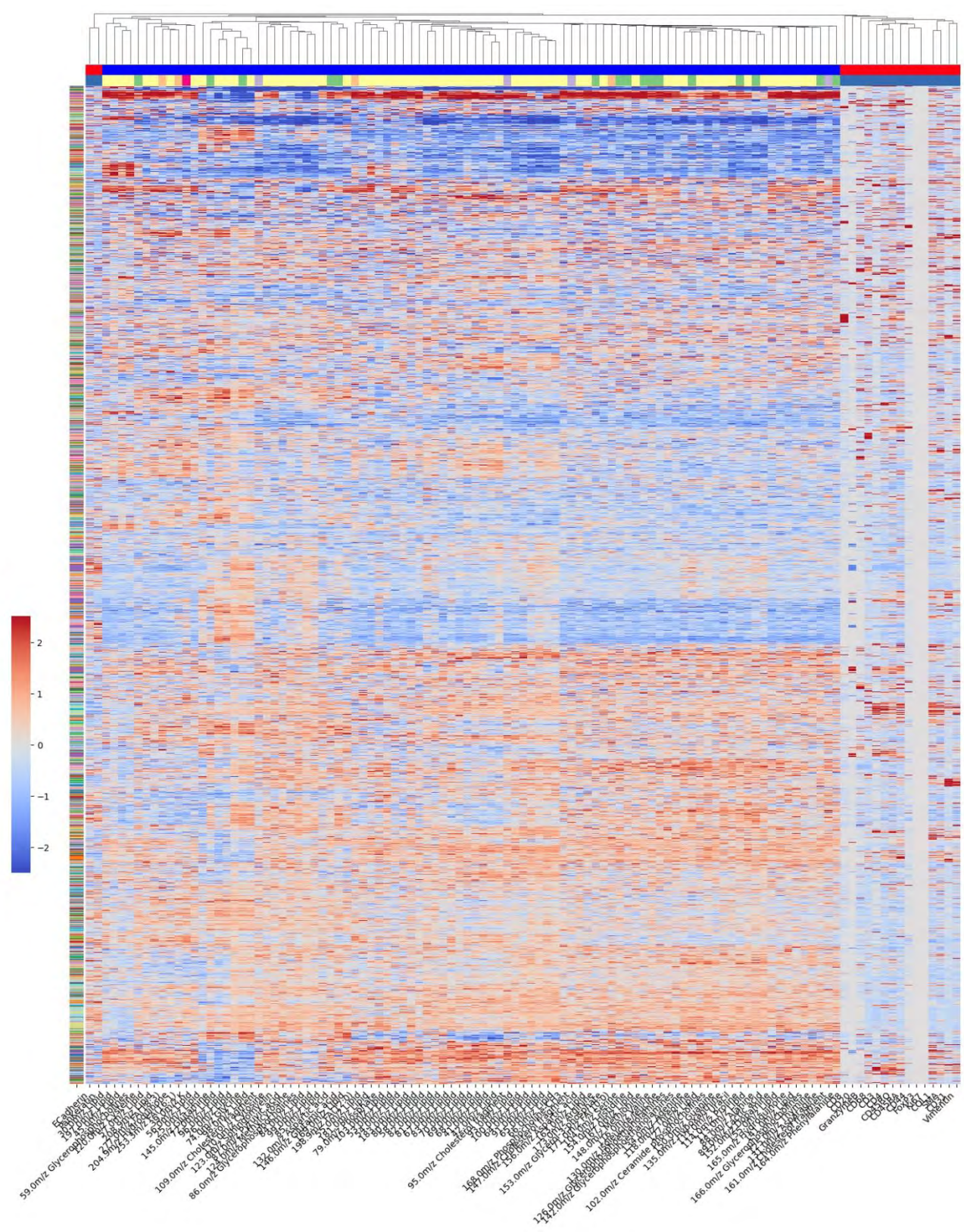
Obese



92 **Supplementary Fig. 10.** Single-cell registration results between IMC Intercalator marker (left) and SIMS PO3-
93 (right) in human endometrium issues. Scale bar 100 μm .

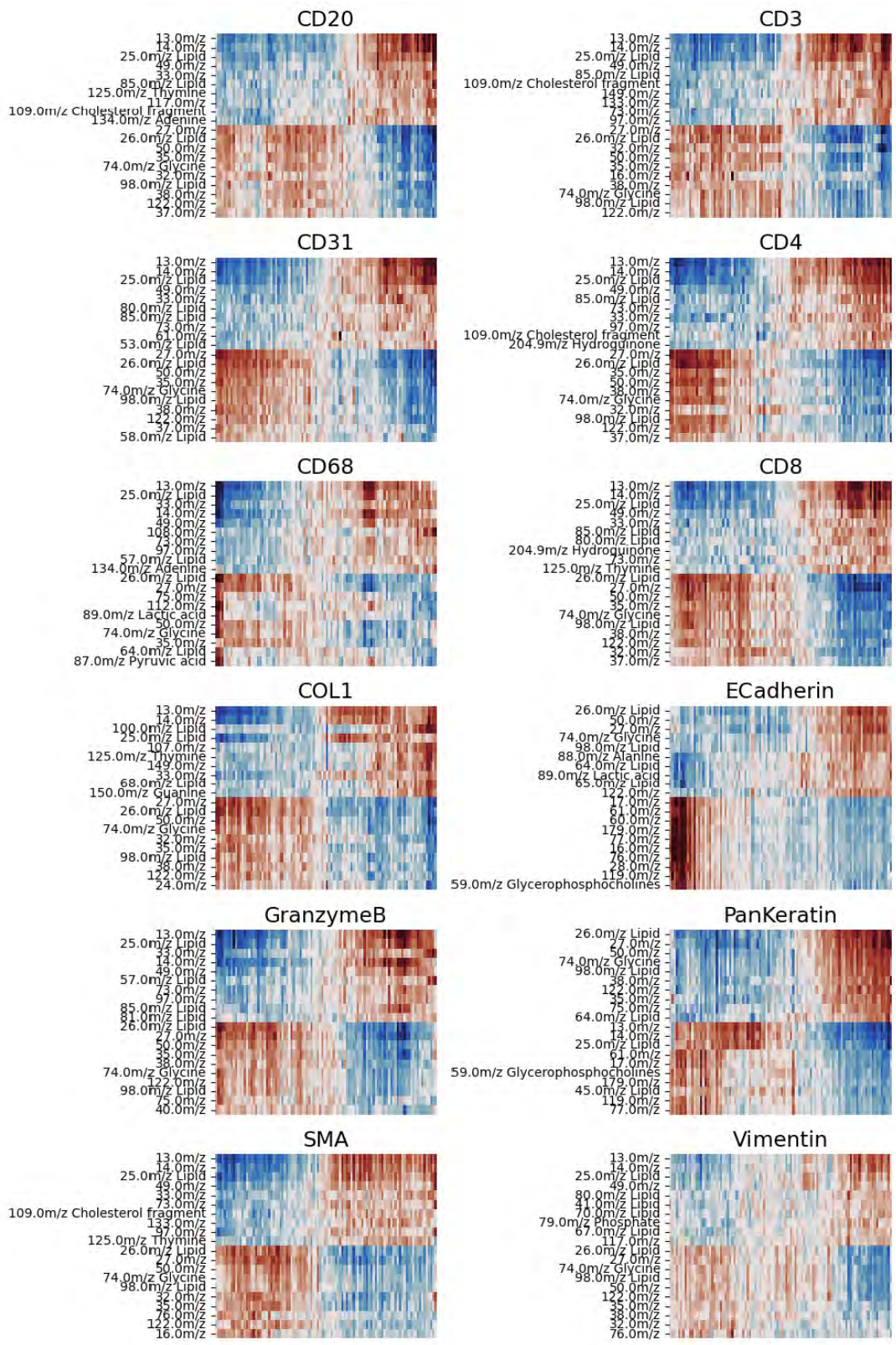
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LUNG

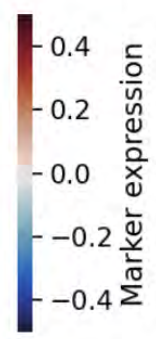


97 **Supplementary Fig. 11.** Single-cell metabolite and protein profile in lung cancer tissues (n=19507 cells). Cluster
98 map showing the cell-level metabolite and protein profile in all lung cancer tissues. Colorbar corresponds to the
99 mean intensity value at the single cell level.

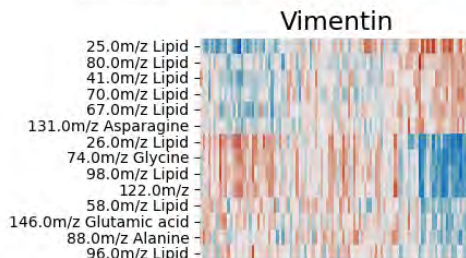
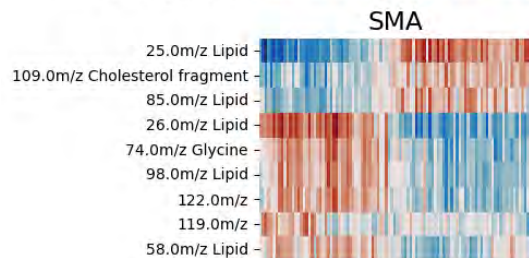
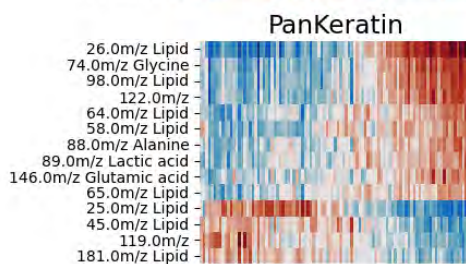
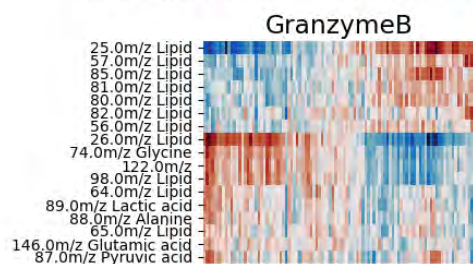
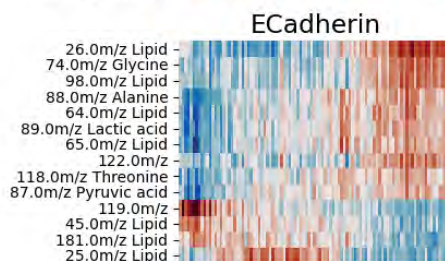
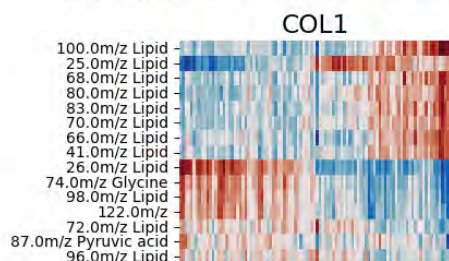
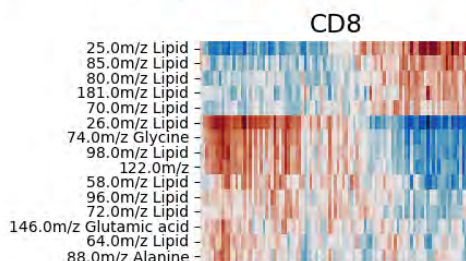
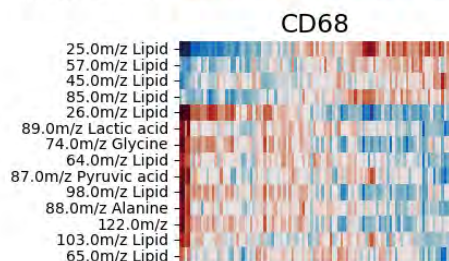
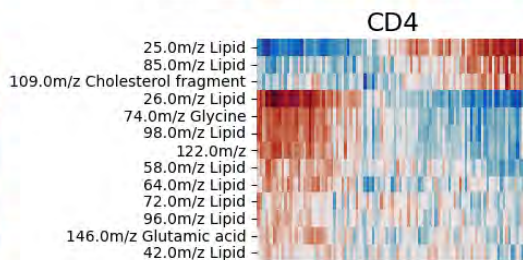
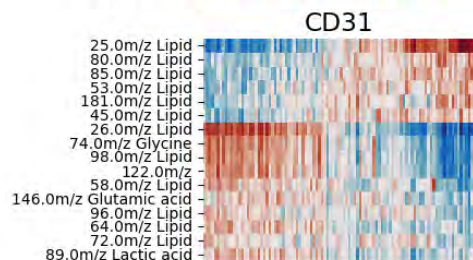
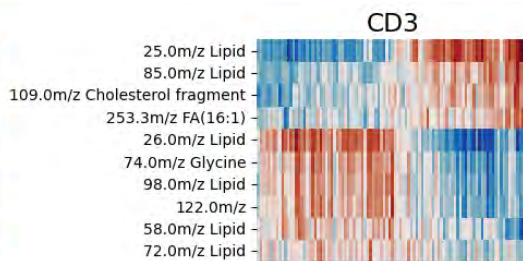
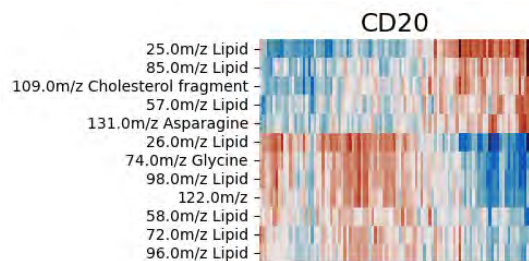
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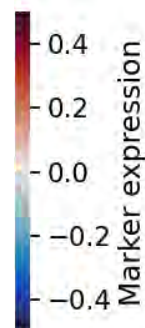
Protein intensity
low to high



Supplementary Fig. 12. Single-cell metabolite and protein profile correlation in lung cancer tissues. Each heatmap shows the correlation of metabolite channels with corresponding protein markers (n=19507 cells). Left to right shows ascending single-cell protein intensity expression.

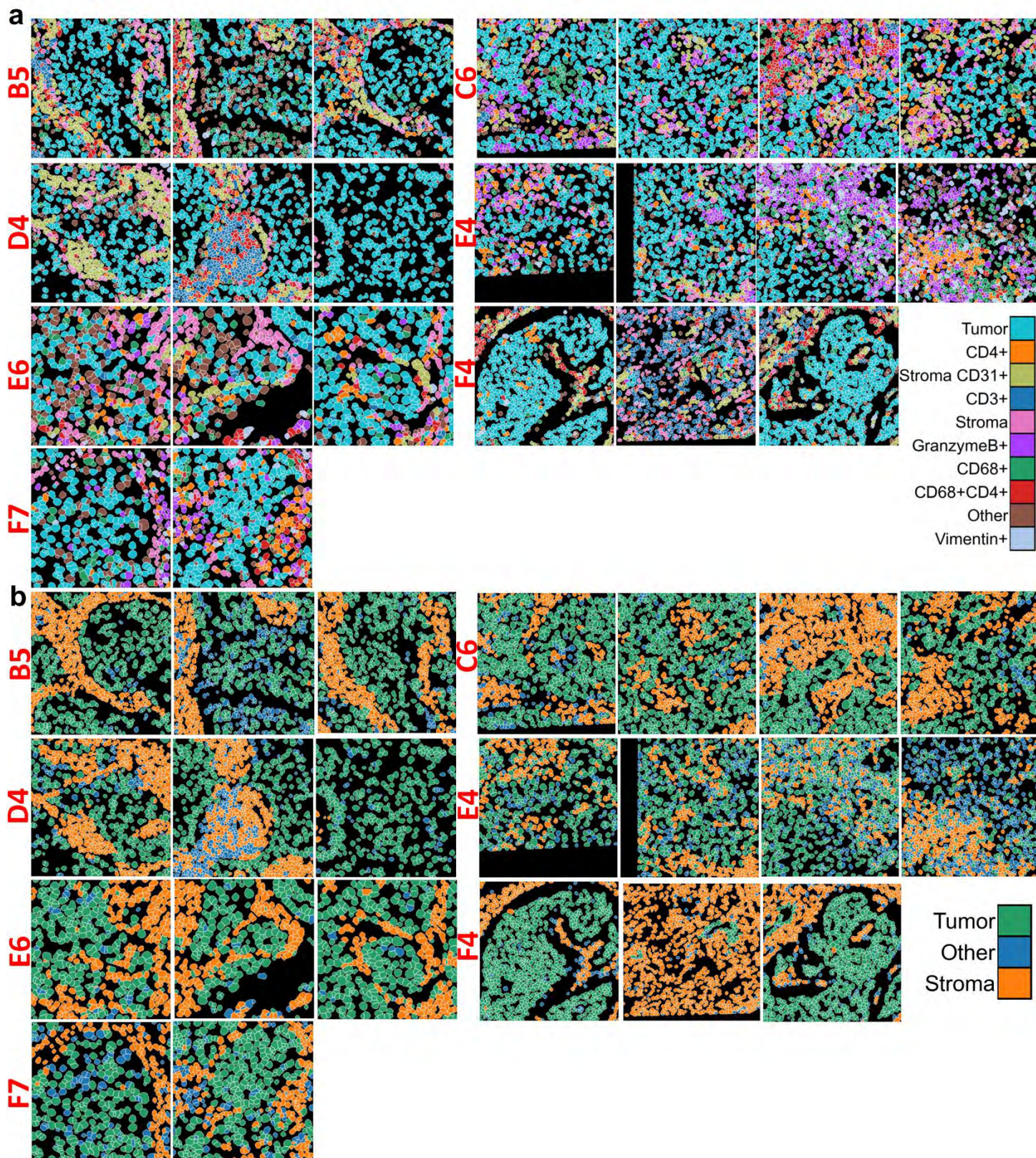


Protein intensity
low to high



108 **Supplementary Fig. 13.** Single-cell metabolite channels associated with glucose, lipid, amino acids, and fatty
109 acids correlated with protein profile in lung cancer tissues. Each heatmap shows the correlation of metabolite
110 channels with corresponding protein markers (n=19507 cells). Left to right shows ascending single-cell protein
111 intensity expression.

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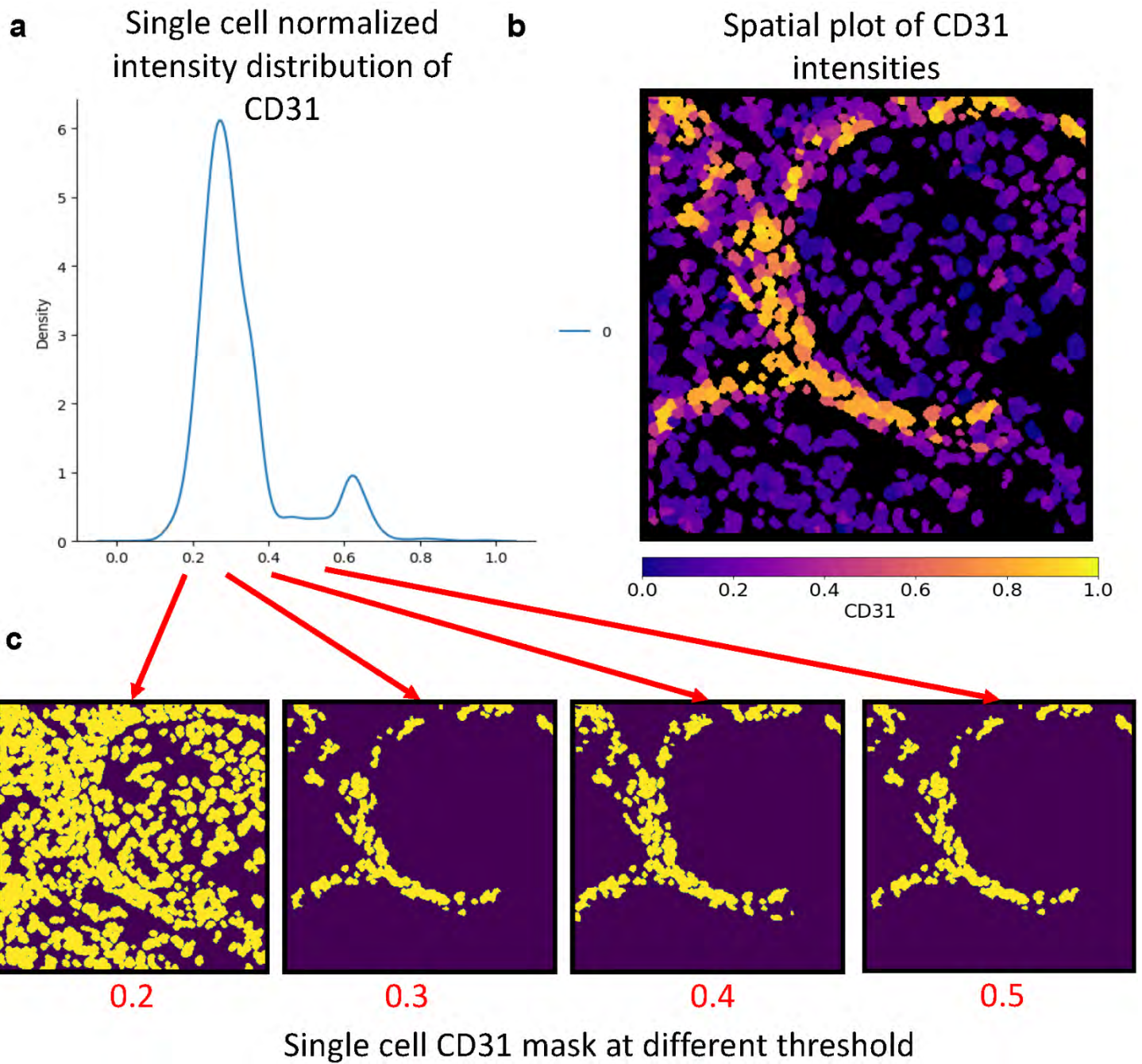


114 **Supplementary Fig. 14.** Single-cell protein phenotyping in cancer lung cancer

115 **a** Spatial projection of unsupervised single cell clusters from protein profiles in human lung cancer tissues
116 for all imaged regions of interest.

117 **b** Classification of cells into the tumor, stroma, and other regions from cell clusters obtained from **a**.

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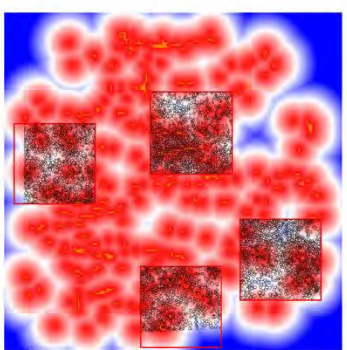
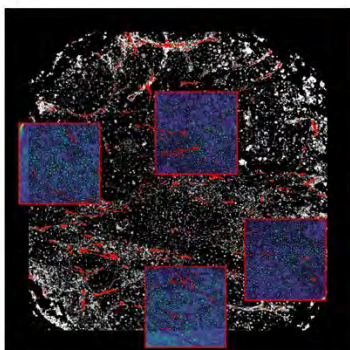
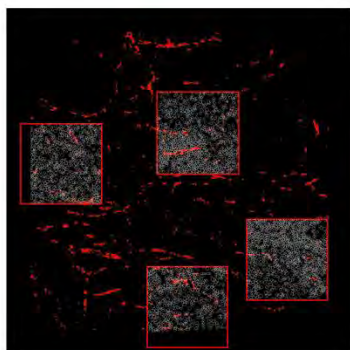
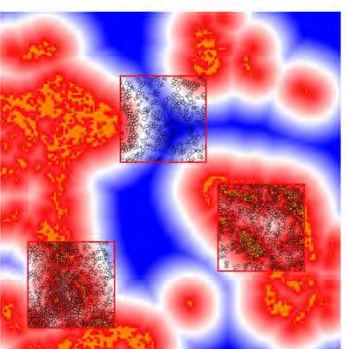
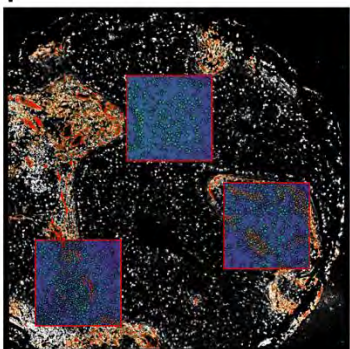
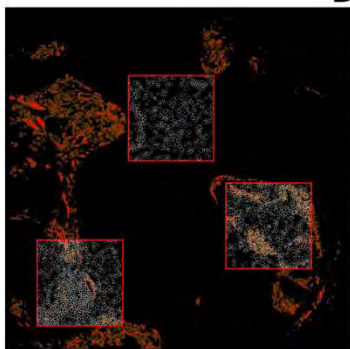
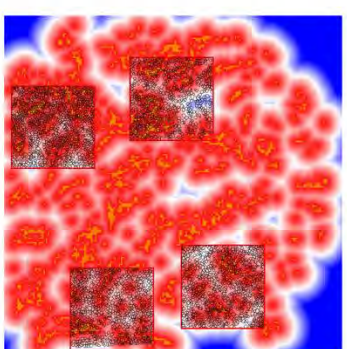
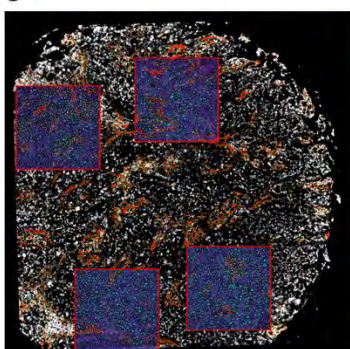
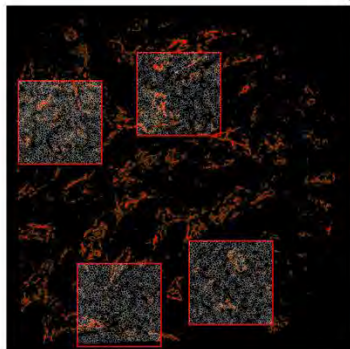
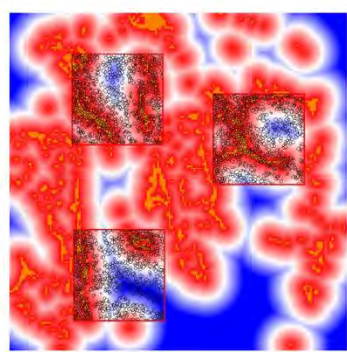
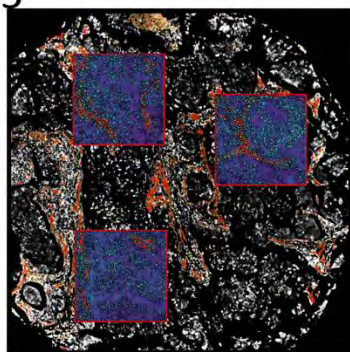
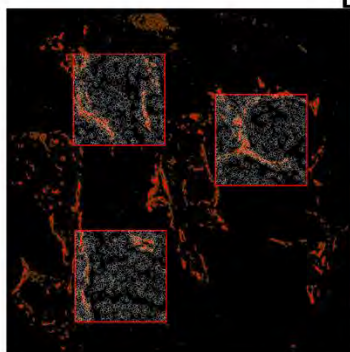


120 **Supplementary Fig. 15.** CD31 marker threshold at the single cell level

121 **a** Bi-modal distribution of single-cell mean intensity of CD31 markers.

122 **b** Corresponding single-cell mask with mean intensity plotted corresponding to the distribution in **a**.

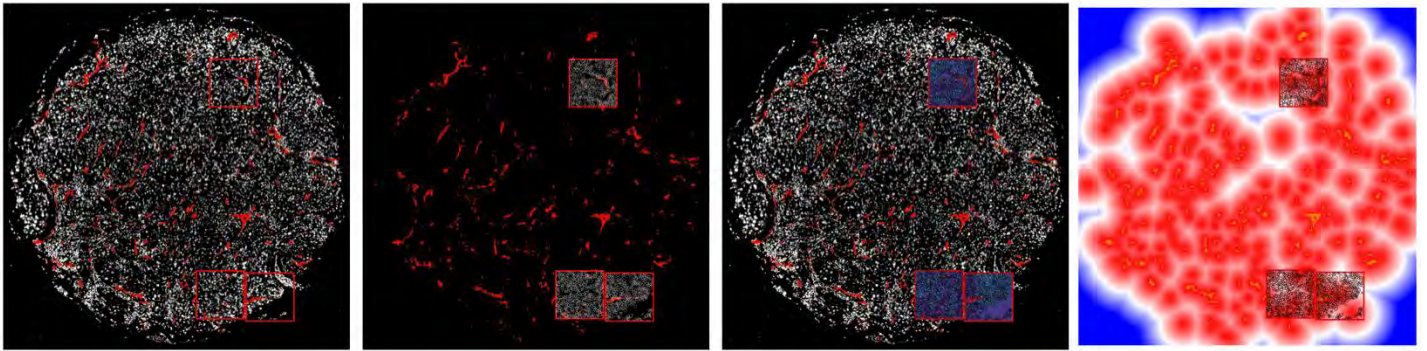
123 **c** Example of CD31 positive single-cell masks at different thresholds: 0.2, 0.3, 0.4 and 0.5.



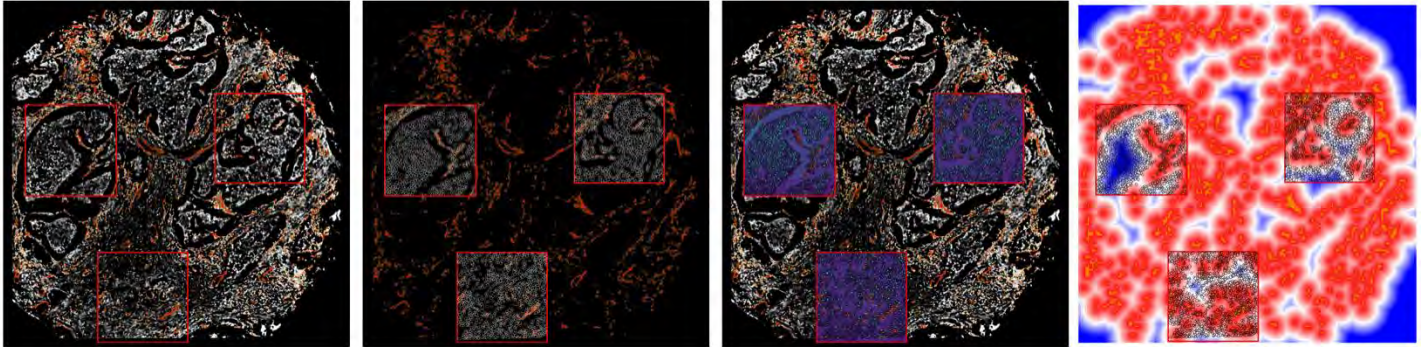
125 **Supplementary Fig. 16.** Single-cell to. CD31+ cell distance matching. Left to right show respectively: IMC
126 multiplex HistoneH3 marker (white) superposed with CD31 marker (red) and corresponding matched 3D-SMF
127 imaged regions showed inside the red bounding box (far left), IMC CD31 marker (red) and corresponding
128 matched 3D-SMF imaged regions in the red bounding box (left center), IMC CD31 marker (red) and
129 corresponding matched PO3- channel from 3D-SMF regions (right center), distance to CD31 positive cell in the
130 spatial domain and corresponding single cell mask in imaged 3D-SMF regions (far right).

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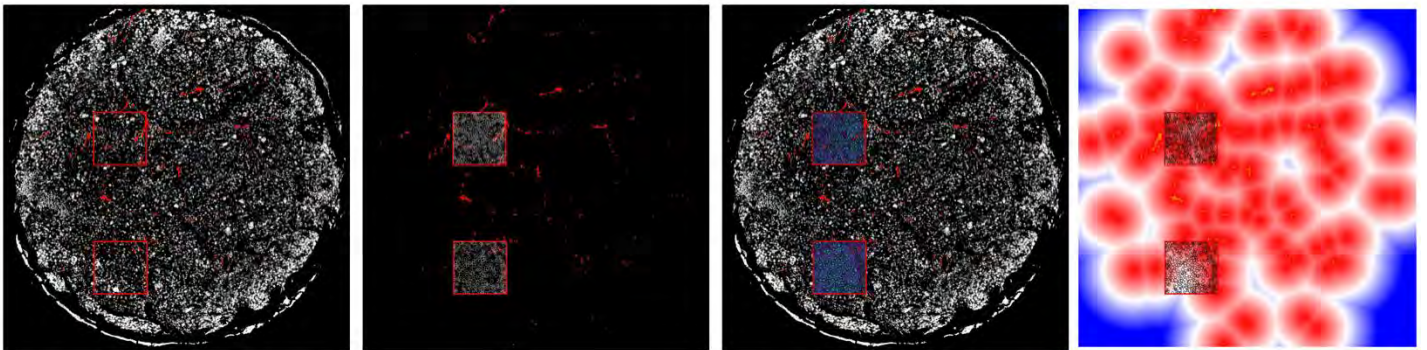
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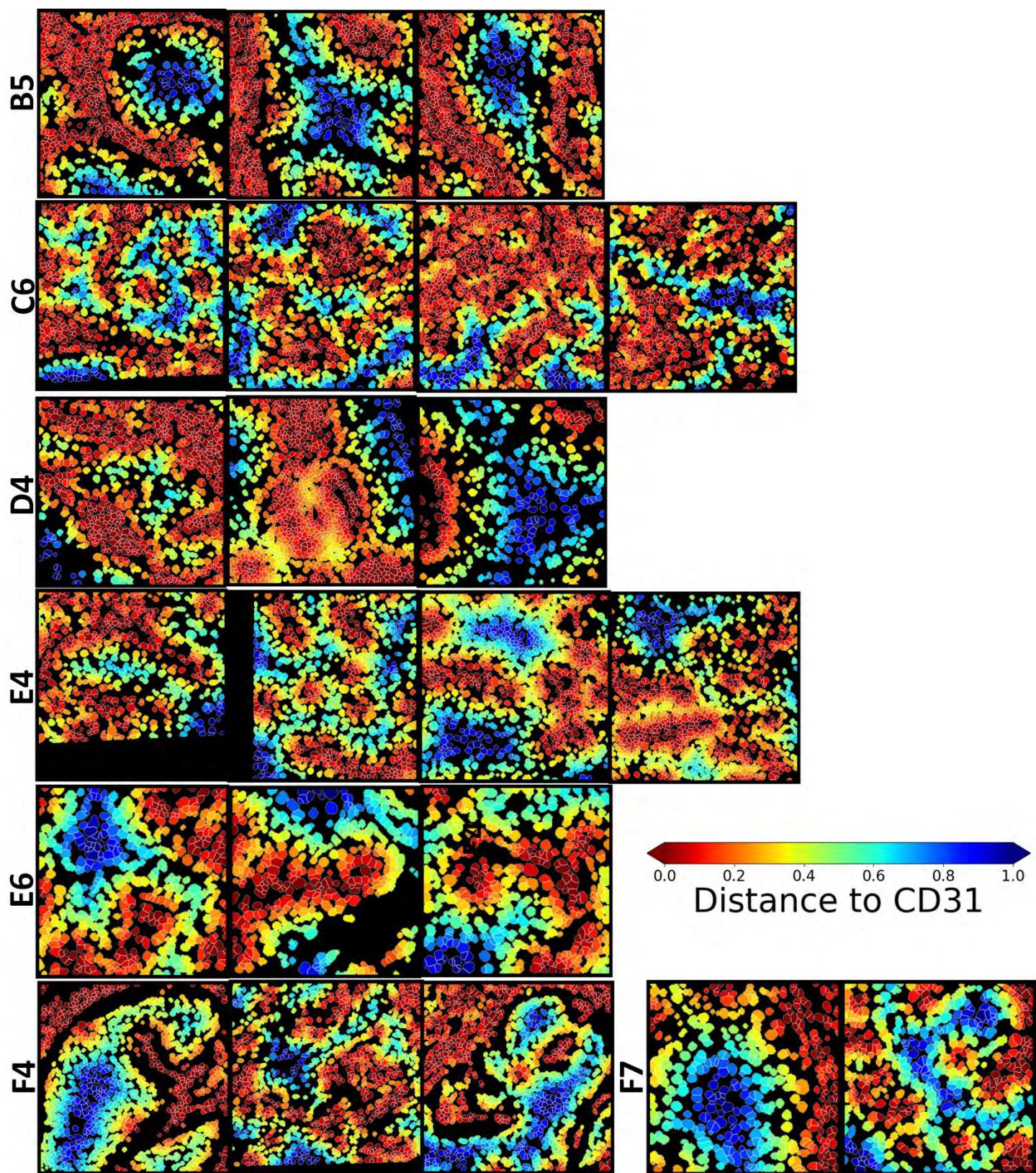
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F7



133 **Supplementary Fig. 17.** Single-cell to. CD31+ cell distance matching. Left to right show respectively: IMC
134 multiplex Histone H3 marker (white) superposed with CD31 marker (red) and corresponding matched 3D-SMF
135 imaged regions showed inside the red bounding box (far left), IMC CD31 marker (red) and corresponding
136 matched 3D-SMF imaged regions in the red bounding box (left center), IMC CD31 marker (red) and
137 corresponding matched PO3- channel from 3D-SMF regions (right center), distance to CD31 positive cell in the
138 spatial domain and corresponding single cell mask in imaged 3D-SMF regions (far right).

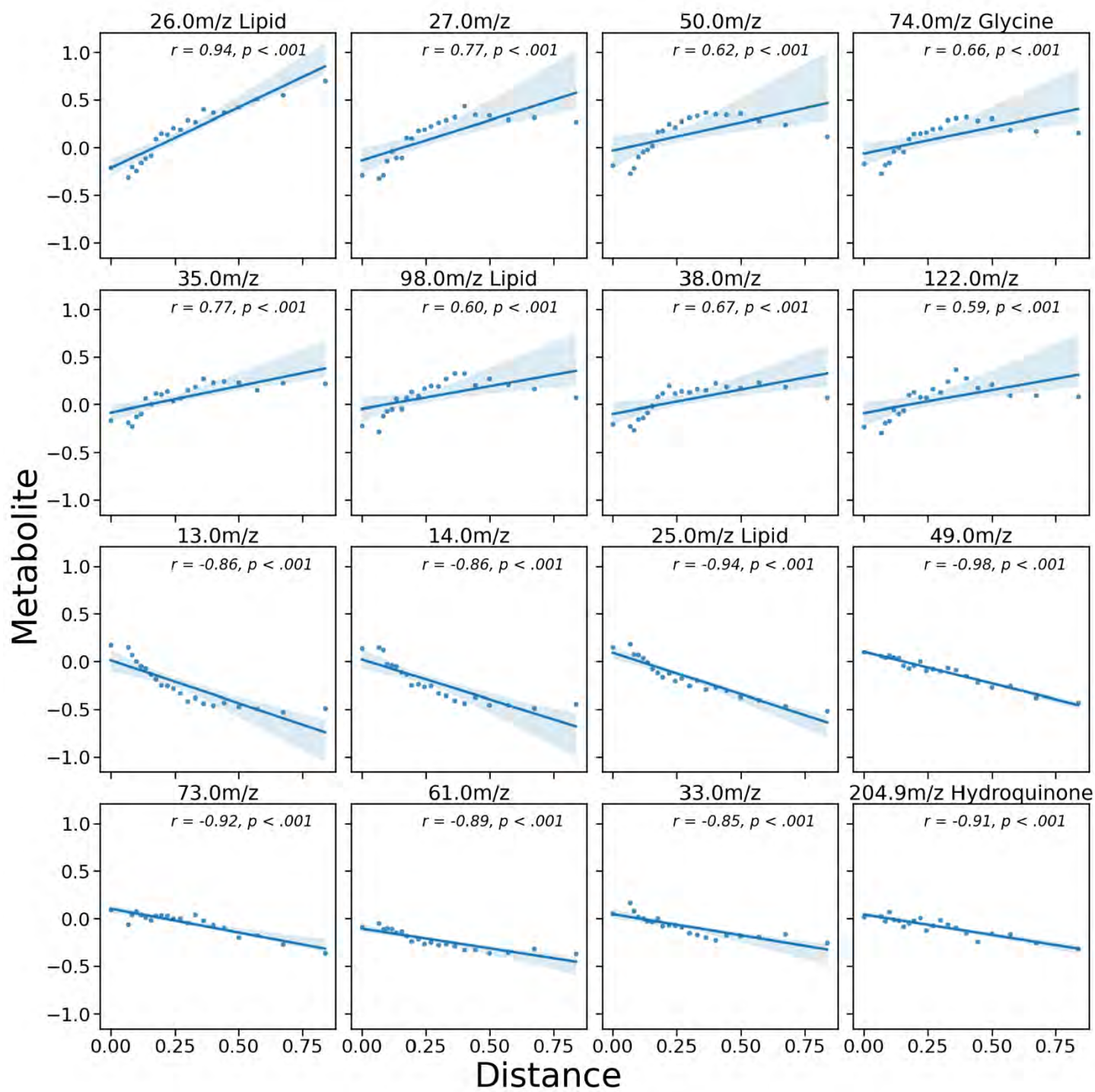


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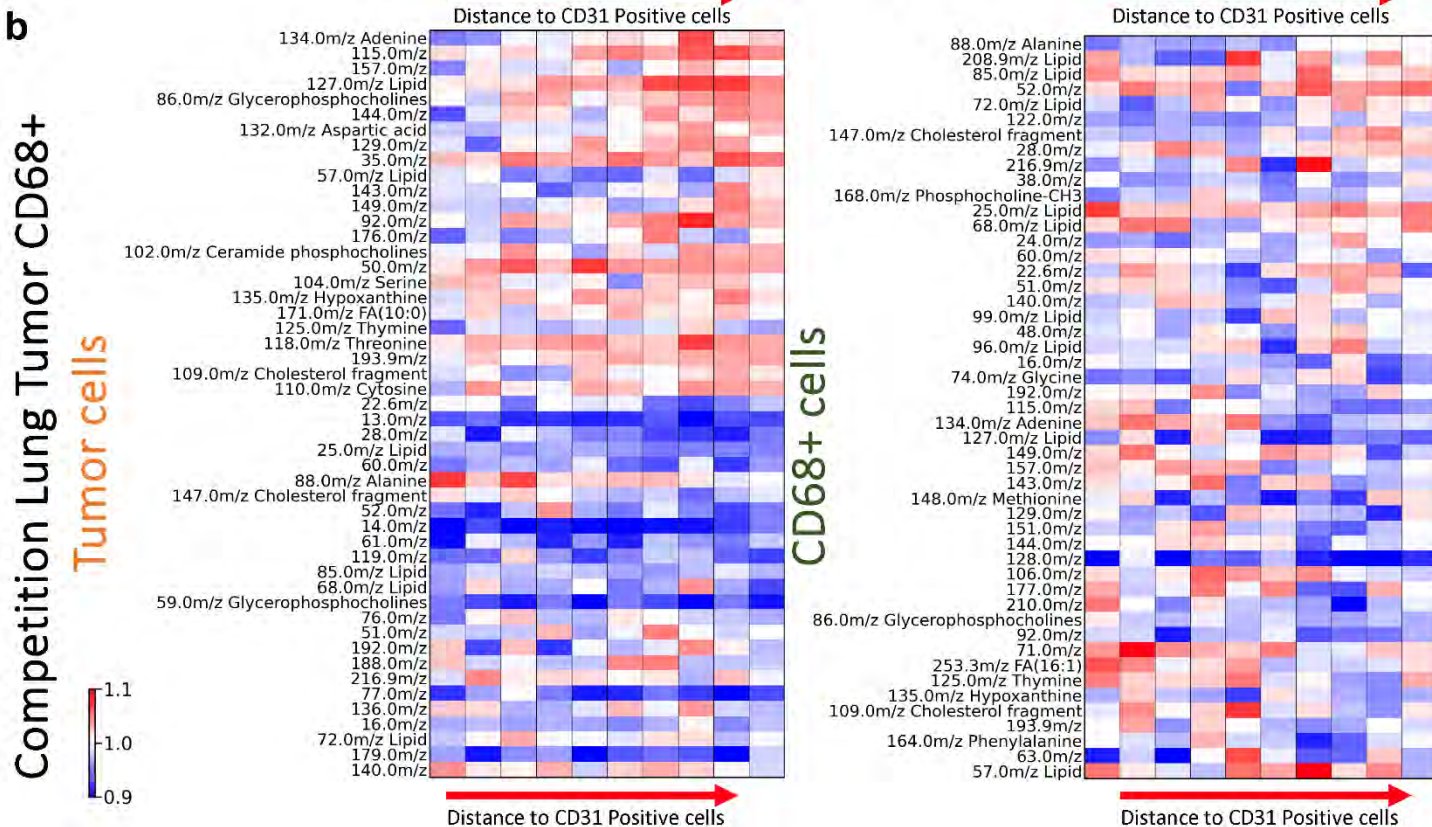
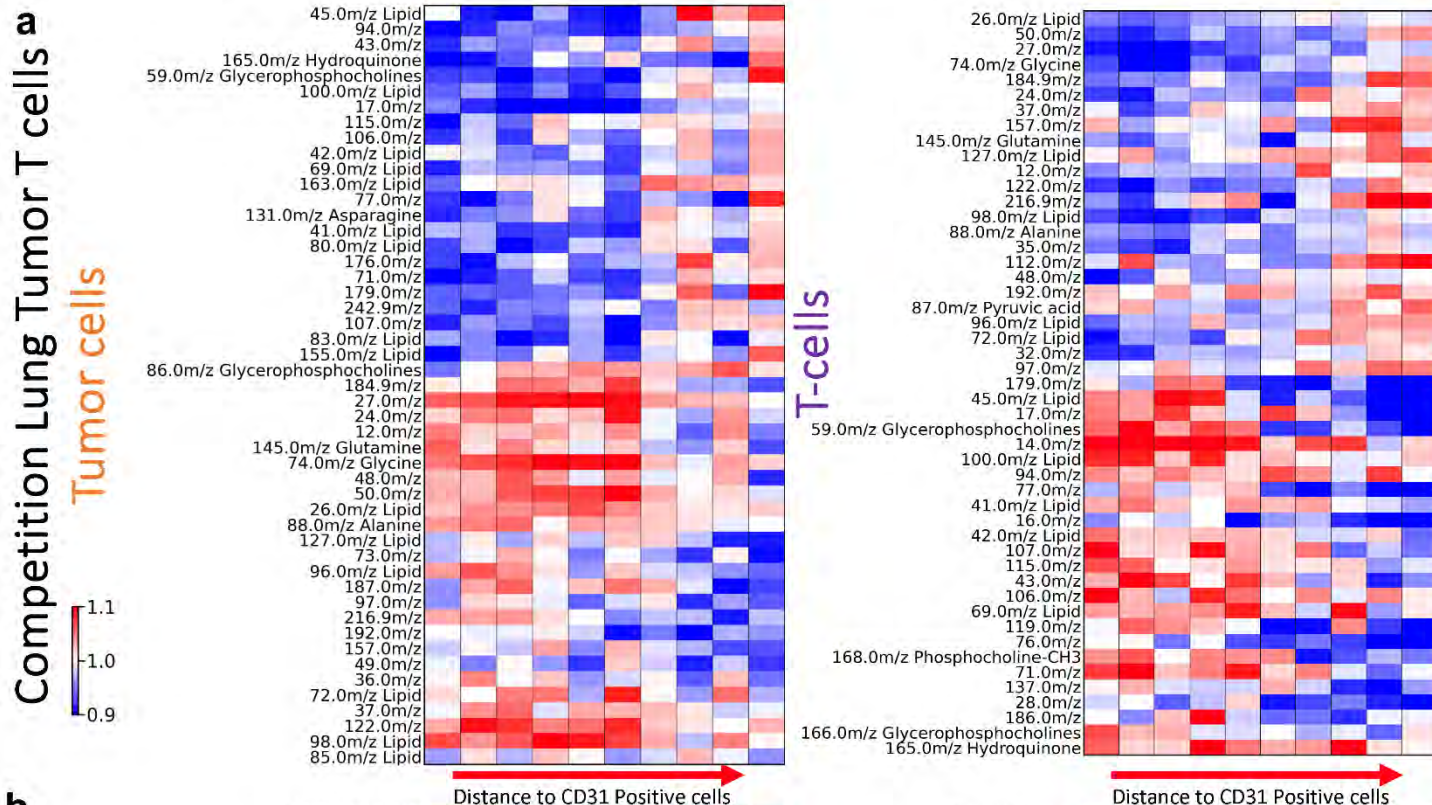
141 **Supplementary Fig. 18.** Single-cell CD31 cells distance map in lung cancer tissues. Single-cell masks in lung
142 cancer tissues with corresponding distance to CD31+ cells overlaid.

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146 **Supplementary Fig. 19.** Correlation plot of most and least correlated metabolite channels with distance to CD31
147 cells. Pearson correlation and p-value calculated from 25 bins distance defined with n=19507 cells.

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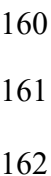


151 **Supplementary Fig. 20.** Single-cell metabolites competition as distance to CD31 positive cells in lung cancer
152 tissues.

153 **a** Local metabolites competition as a distance of CD31+ cells between T-cells and tumor cells (n = 6536
154 cells). Selected metabolite channels showing positive and negative correlation of tumor cells (left) and T-
155 cells (right). Colorbar shows the metabolites competition ratio.

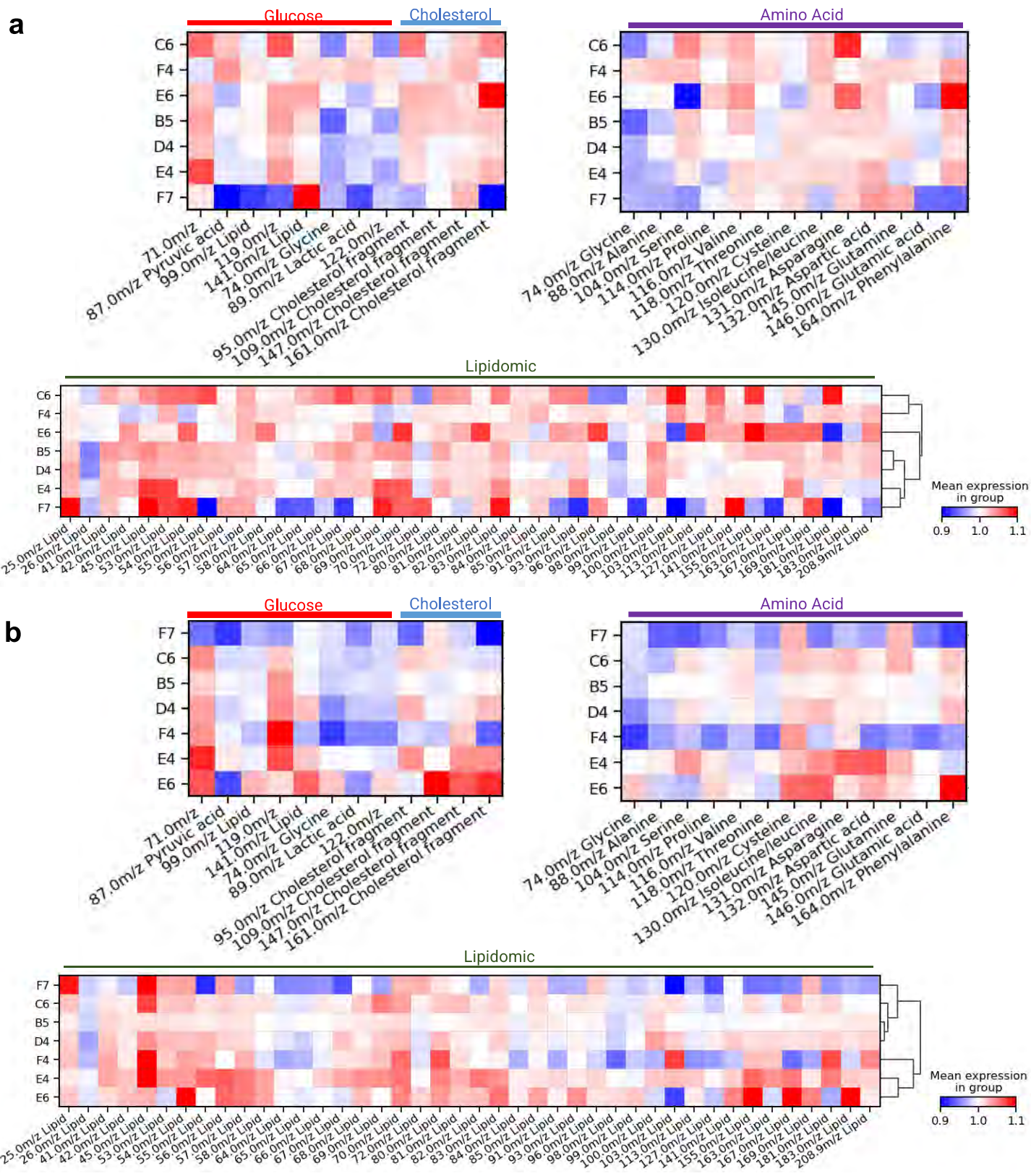
156 **b** Local metabolites competition as a distance of CD31+ cells between CD68+ cells and tumor cells (n =
157 8130 cells). Selected metabolite channels showing positive and negative correlation of tumor cells (left)
158 and CD68+ cells (right). Colorbar shows the metabolites competition ratio.

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Supplementary Fig. 21. ScSpaMet stratifies lung cancer patients and shows cell-specific metabolite states.

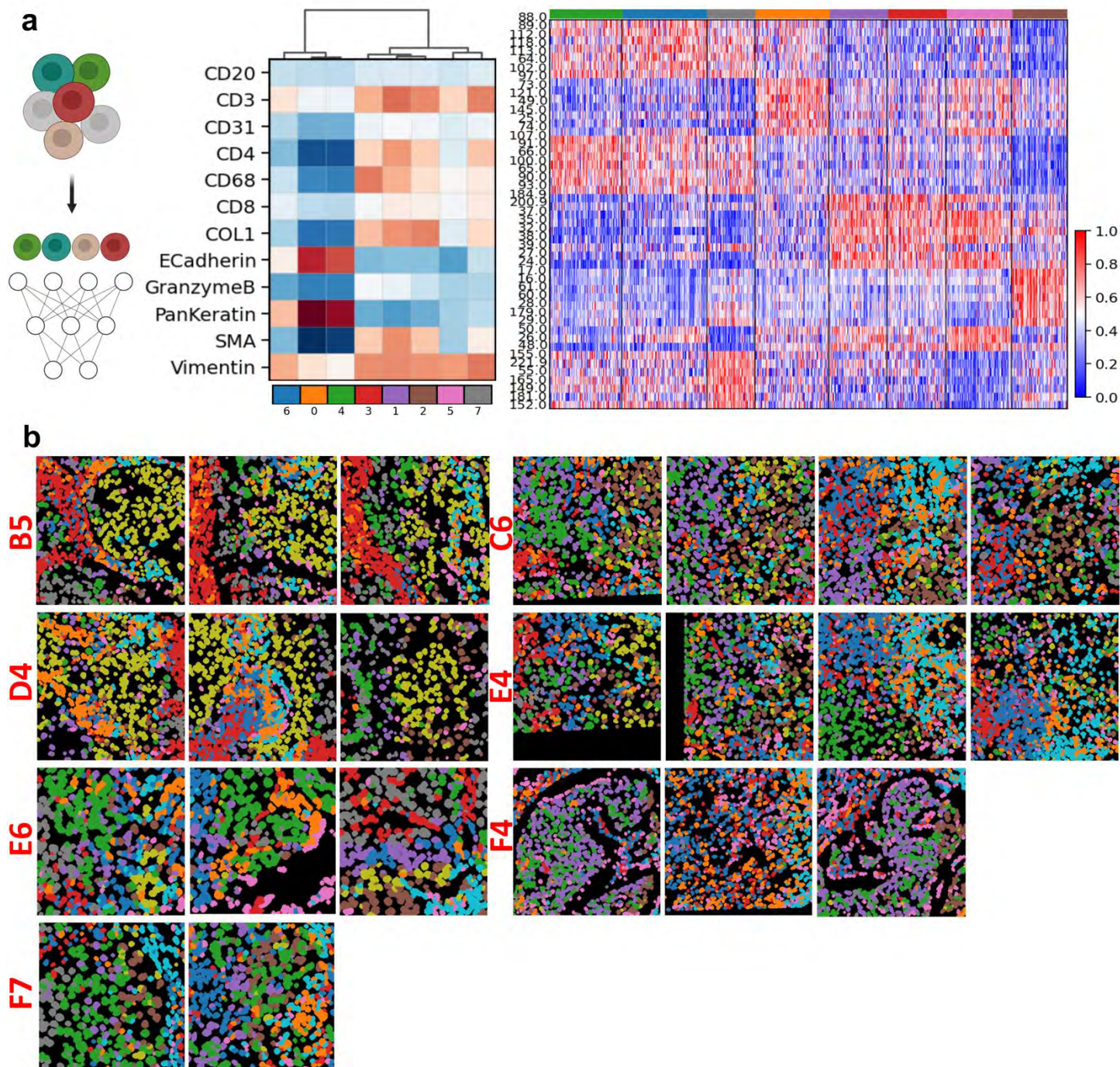
- a** Representative schematic showing the single cell metabolites comparison across patients in lung tumor tissues. Created with Biorender.com
- b** Dot plot showing high variation (top) and low variation (bottom) metabolite channels across patients in lung tumor tissues.
- c** VAE single cell protein-metabolite joint embedding showing stratification of patients and joint protein-metabolites single cell phenotype. Created with Biorender.com



173 **Supplementary Fig. 22** Single-cell joint metabolite competition for each TMA core for **a** Tumor and T-cells (n
174 = 6536 cells) and **b** Tumor and CD68⁺ cells (n = 8130 cells).

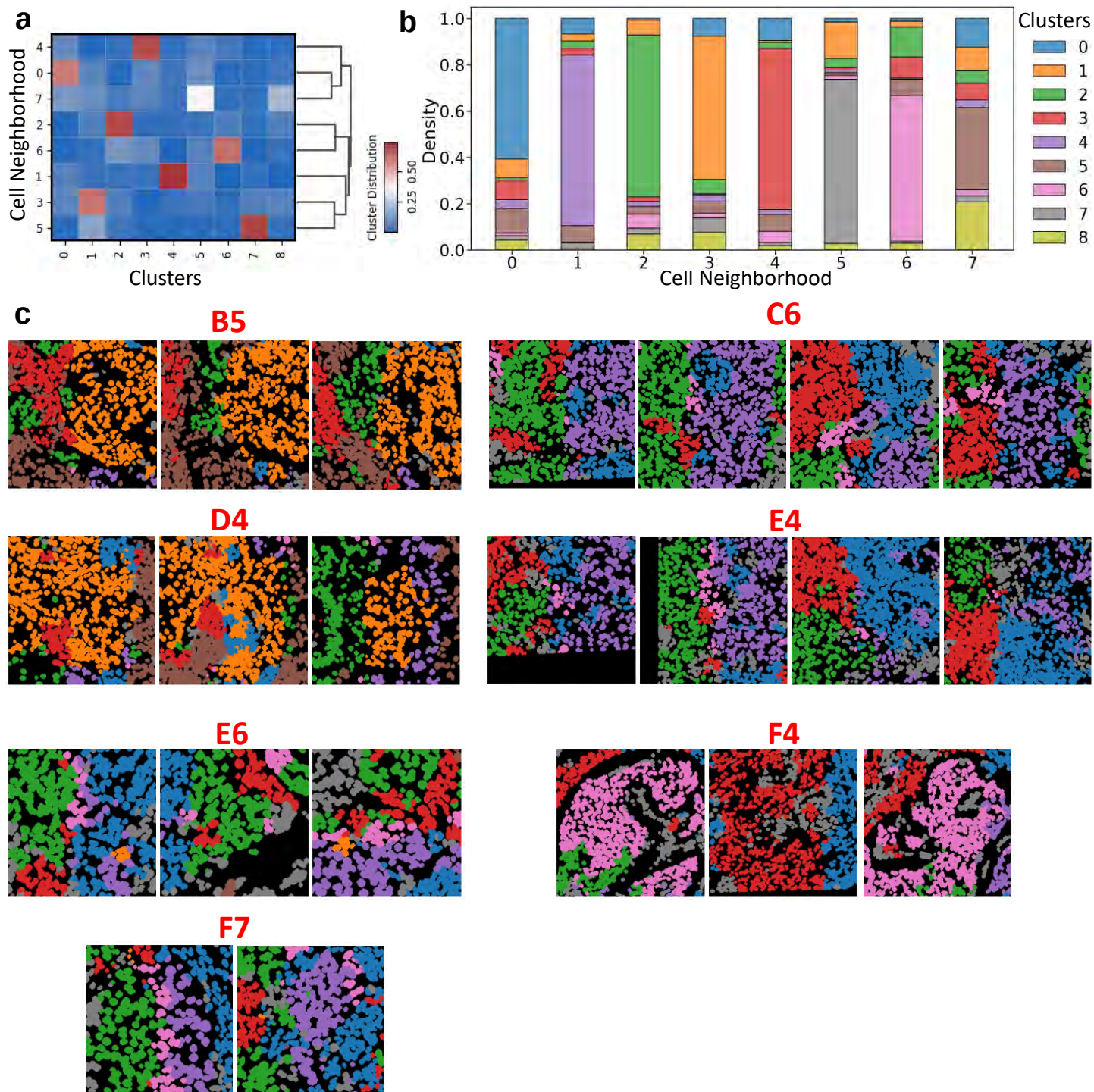
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Supplementary Fig. 23. Single-cell joint protein-metabolite phenotyping in lung cancer.

- a** VAE single cell protein-metabolite joint embedding. Left: architecture for single-cell protein-metabolite joint embedding (n=19507 cells). Right: joint protein-metabolites single-cell phenotype. The colorbar shows the single cell mean expression value. Created with Biorender.com
- b** Spatial projection of clusters obtained from a) in human lung cancer tissues for all imaged regions of interest.



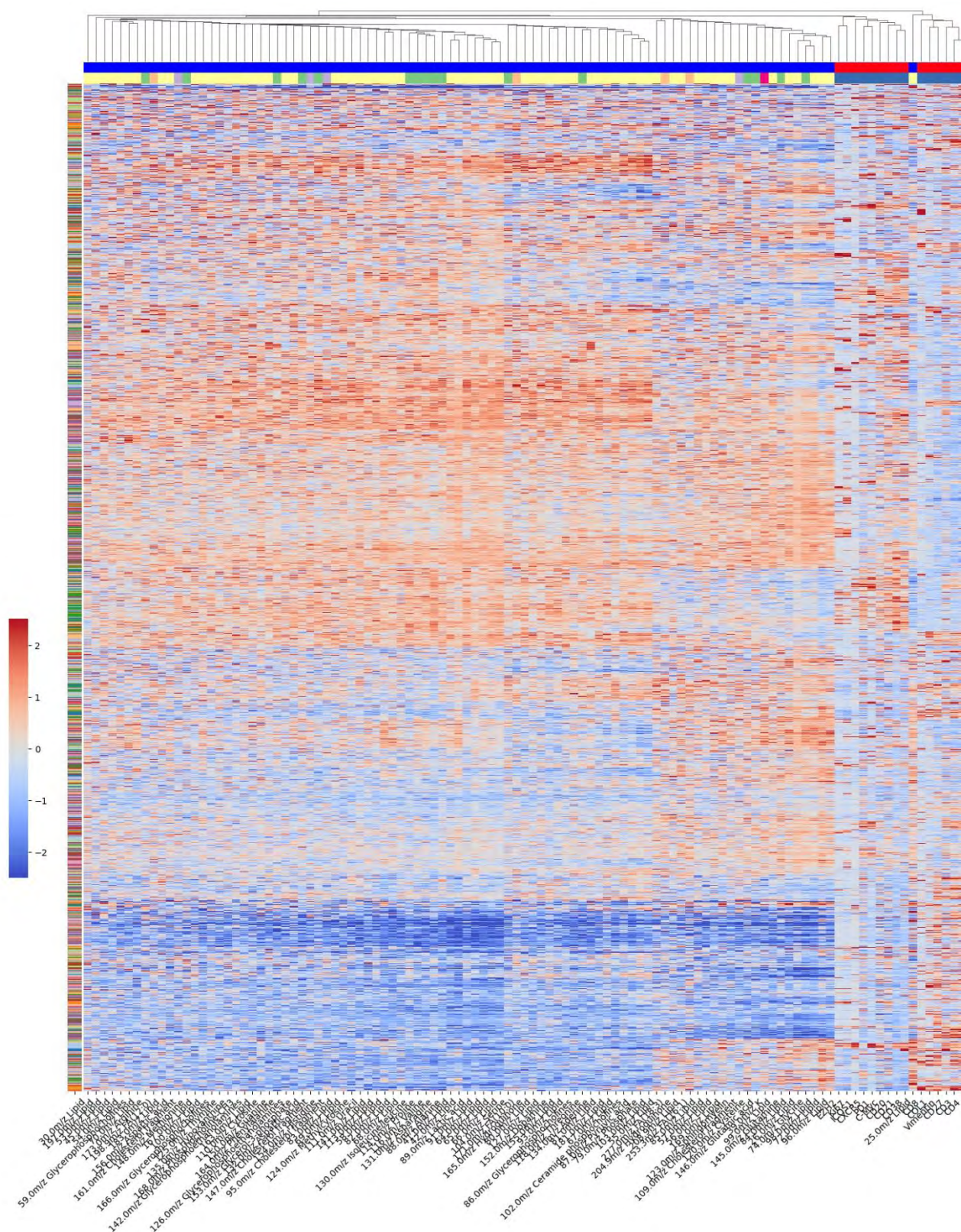
186 **Supplementary Fig. 24.** Single-cell joint protein-metabolite signatures in lung cancer (n=19507 cells).

187 **a** Count of each unsupervised cluster in all the defined cell neighborhoods.

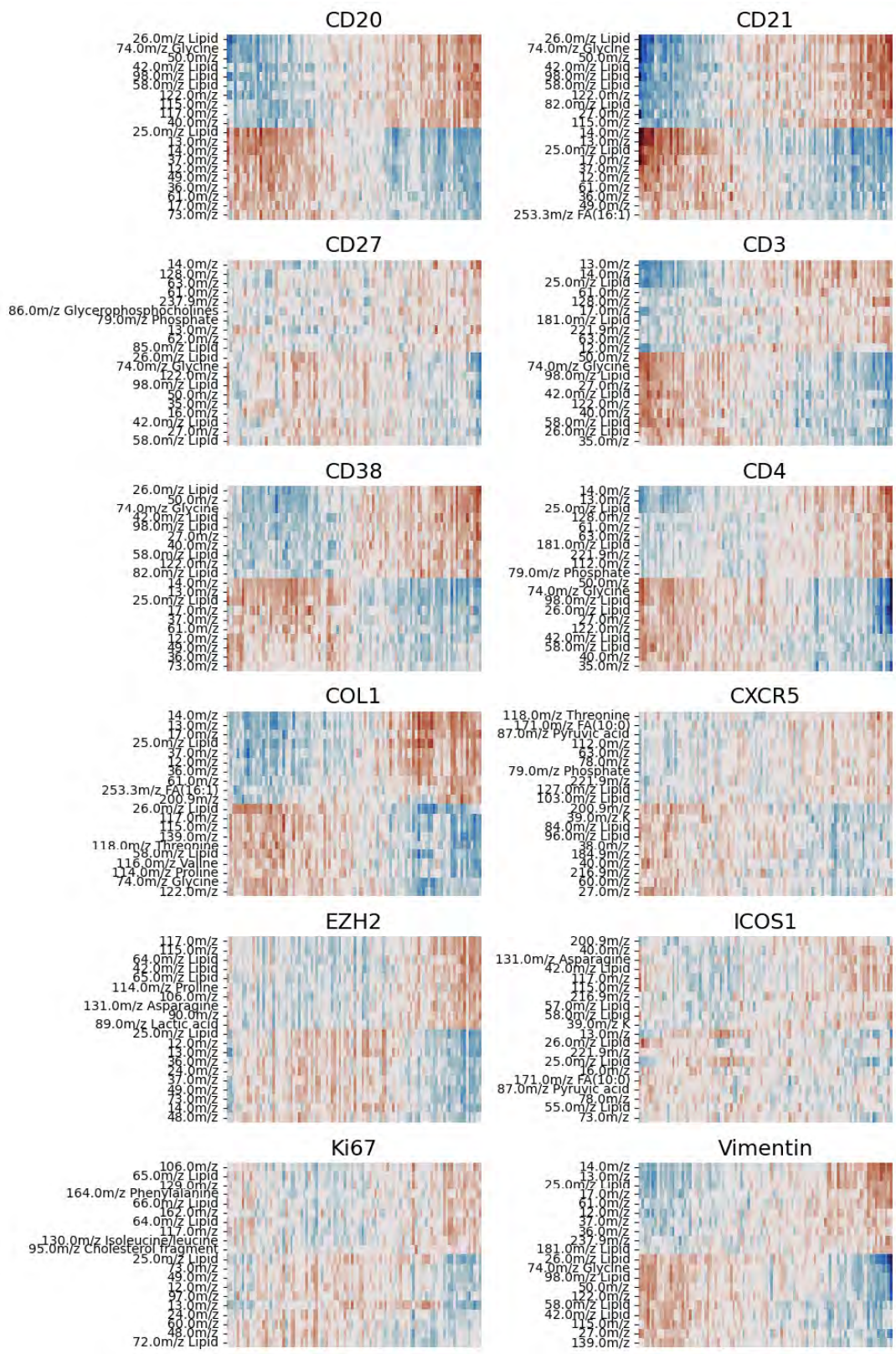
188 **b** The density of each unsupervised cluster in all defined cell neighborhoods.

189 **c** Spatial projection of cell neighborhood obtained from **a** and **b** in human lung cancer tissues for all imaged

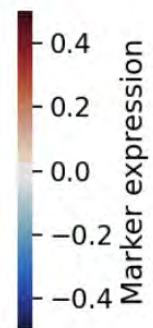
190 regions of interest.



194 **Supplementary Fig. 25.** Single-cell metabolite and protein profile in tonsil tissues (n=31 156 cells). Cluster map
195 showing the cell-level metabolite and protein profile in all tonsil tissues. Color corresponds to the mean intensity
196 value at the single cell level.

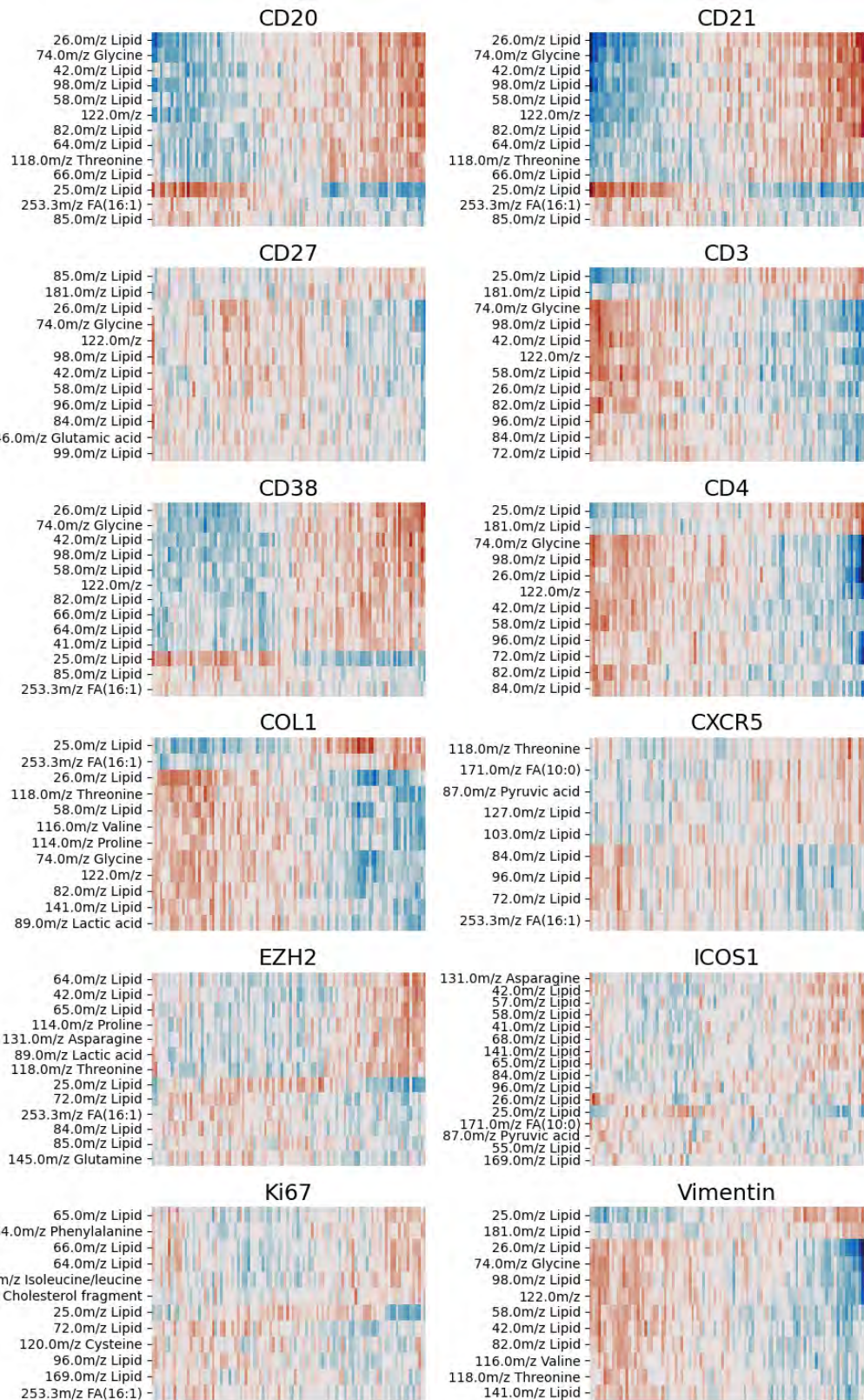


Protein intensity
low to high

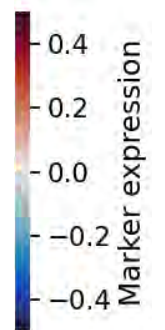


198 **Supplementary Fig. 26.** Single-cell metabolite and protein profile correlation in tonsil tissues. Each heatmap
199 shows the correlation of metabolite channels with corresponding protein markers (n=31156 cells). Left to right
200 shows ascending single-cell protein intensity expression.

201

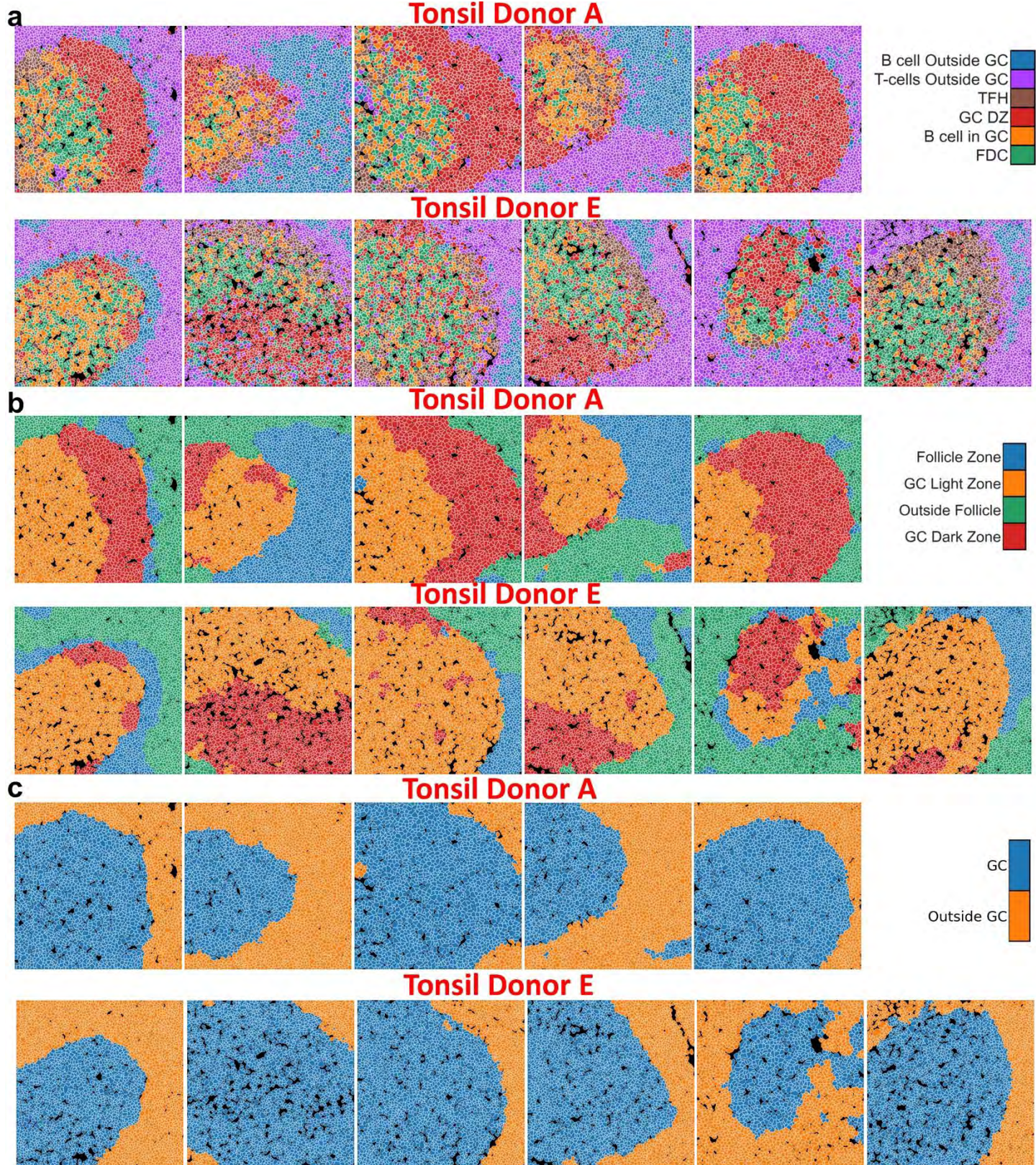


Protein intensity
low to high



203 **Supplementary Fig. 27.** Single-cell metabolite channels associated with glucose, lipid, amino acids, and fatty
204 acids correlated with protein profile in tonsil tissues (n=31156 cells). Each heatmap shows the correlation of
205 metabolite channels with corresponding protein markers. Left to right shows ascending single-cell protein
206 intensity expression.

207



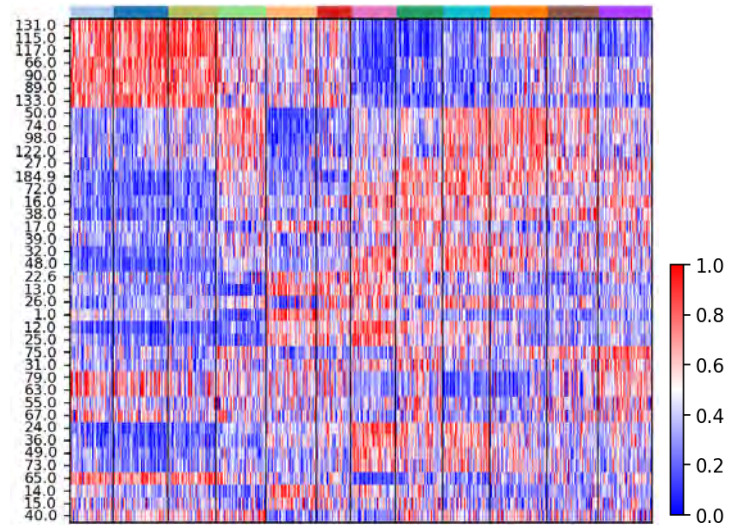
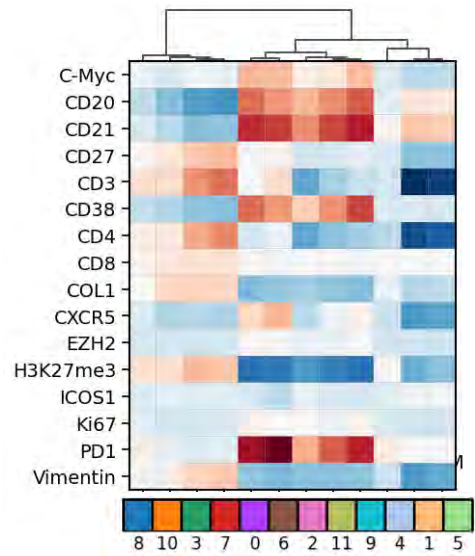
208

209

Supplementary Fig. 28. Single-cell protein phenotyping in human tonsils.

- a** Spatial projection of unsupervised single-cell clusters from protein profiles in human tonsil tissues for all imaged follicle regions.
- b** Classification of cells into the follicle, outside follicle, germinal center dark zone, and germinal center light zone regions from cell clusters obtained from a).
- c** Classification of cells into germinal center dark and non-germinal center regions from cell clusters obtained from a)

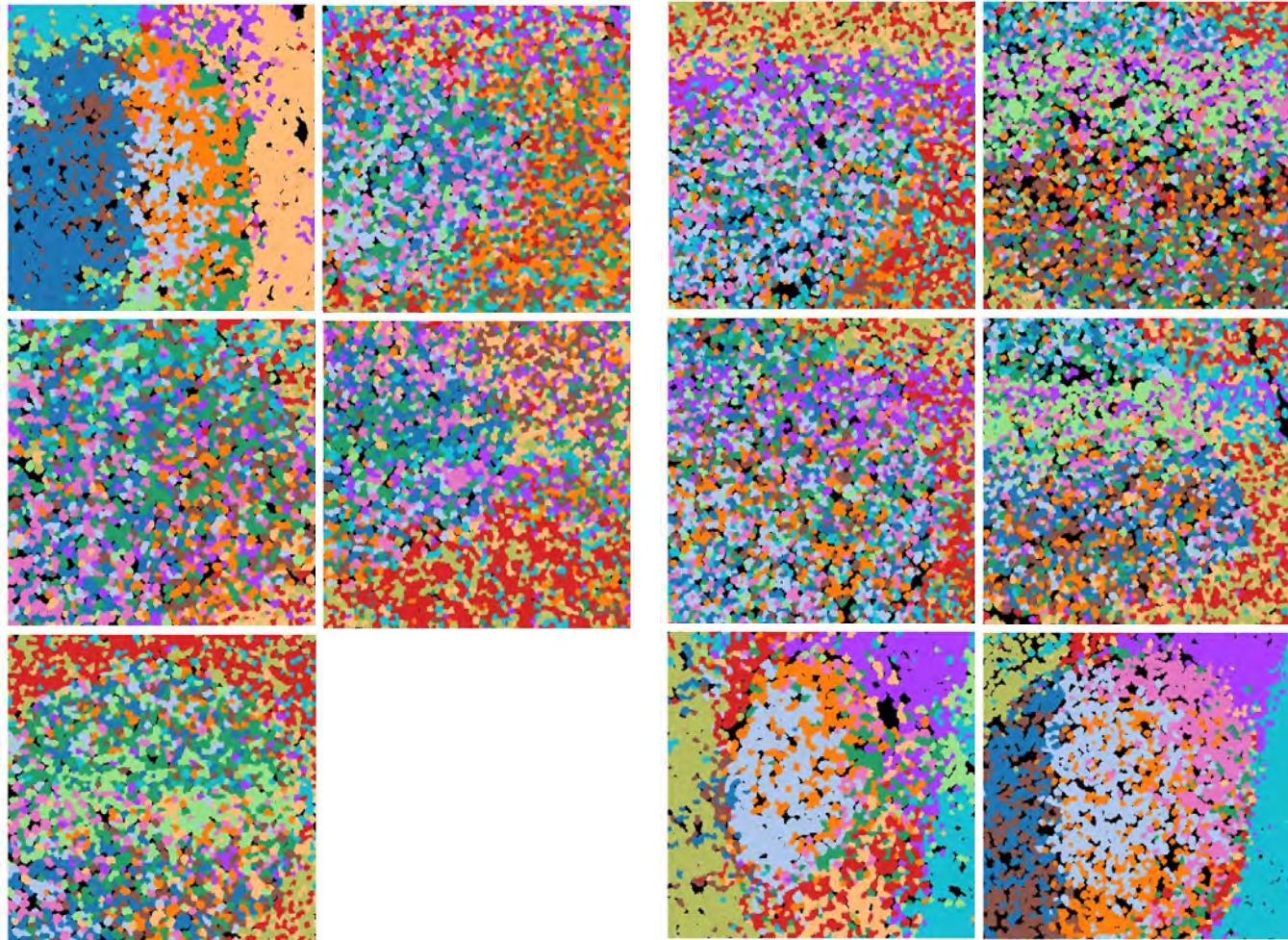
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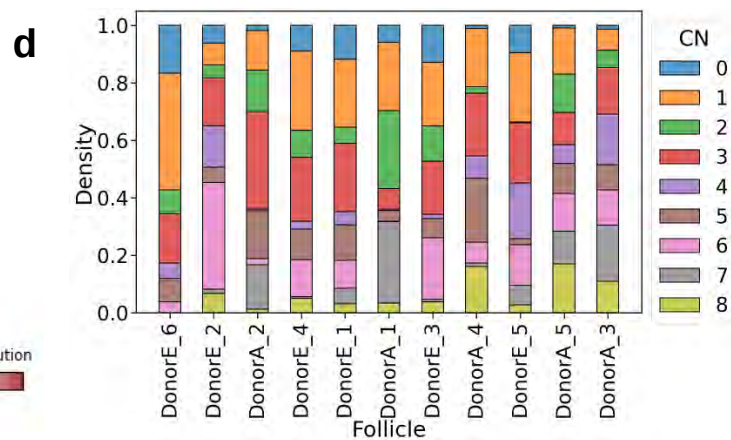
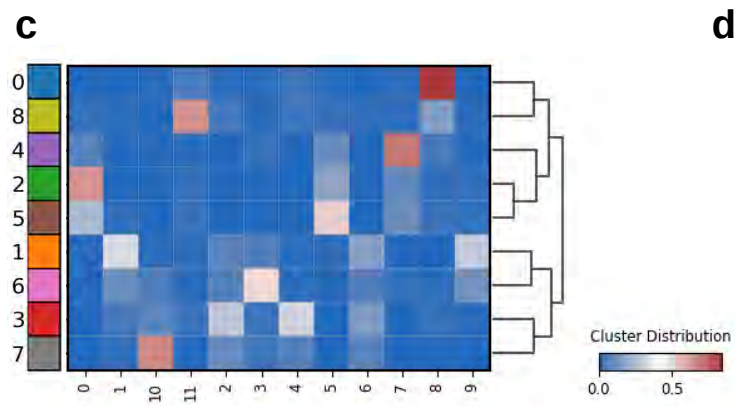


b

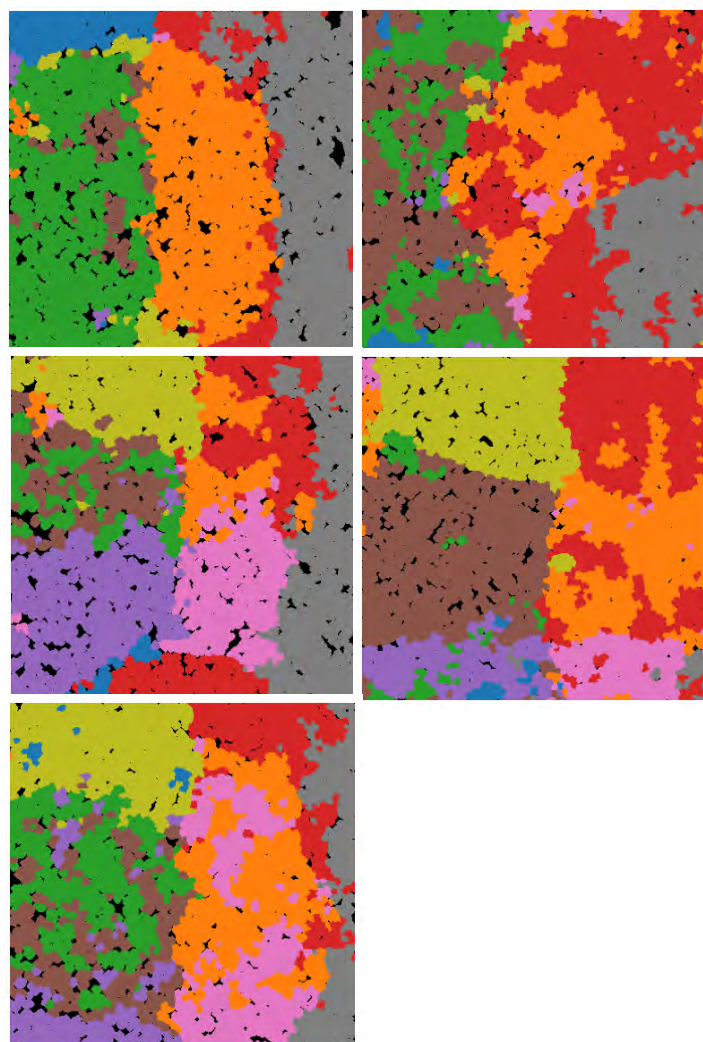
Tonsil Donor A

Tonsil Donor E

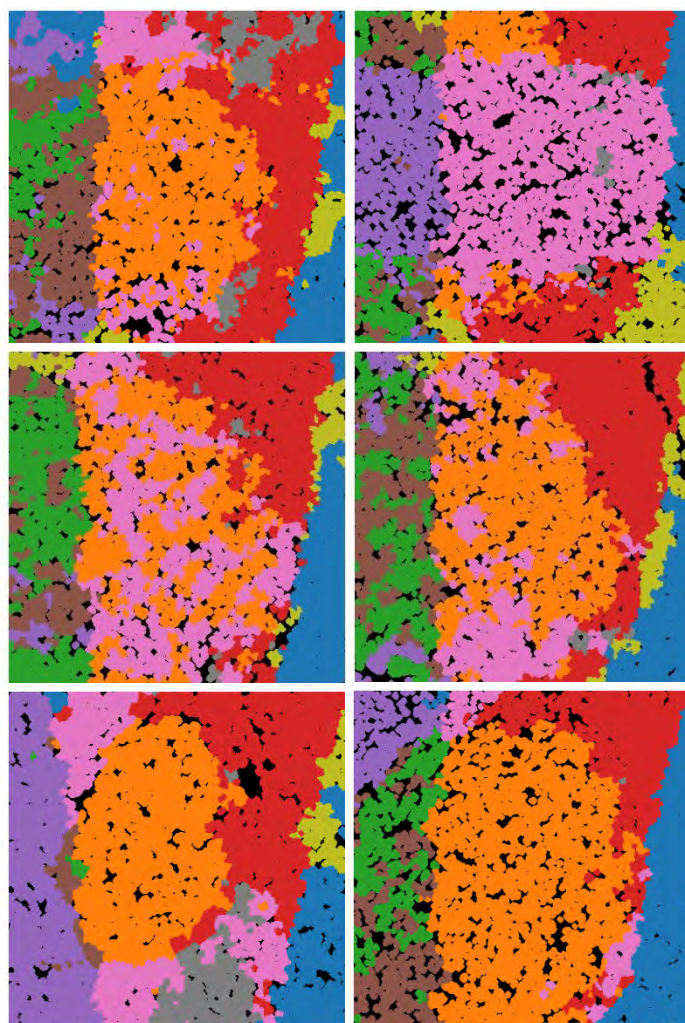




e **Tonsil Donor A**

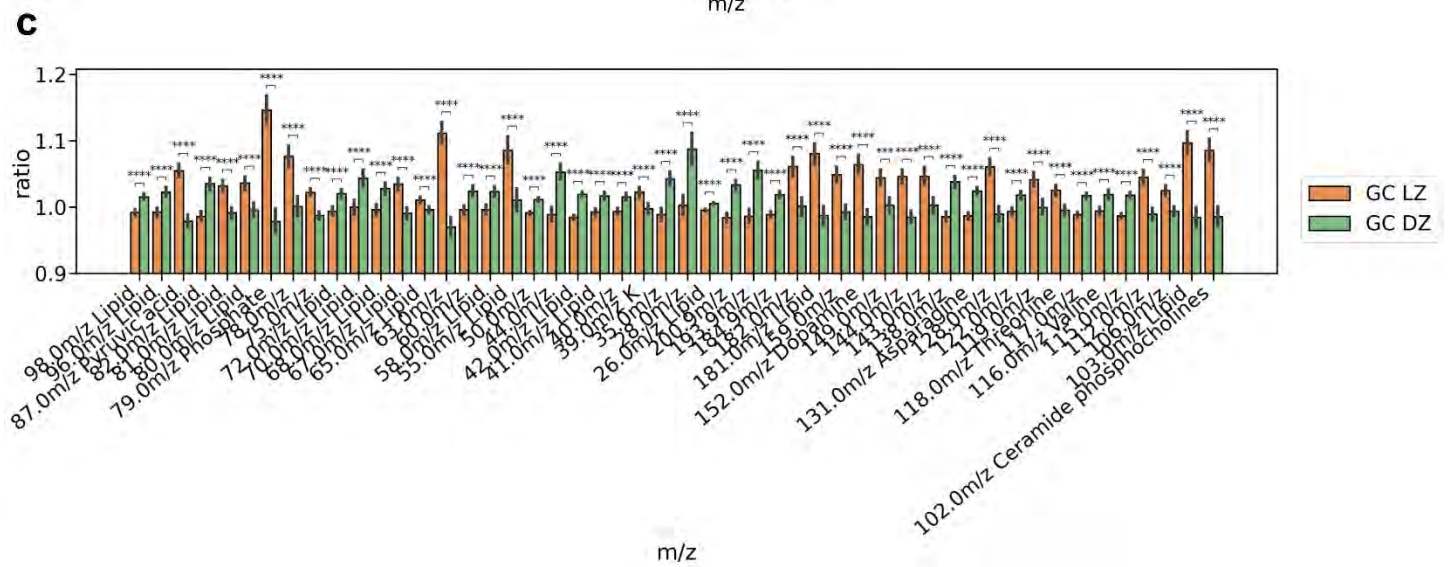
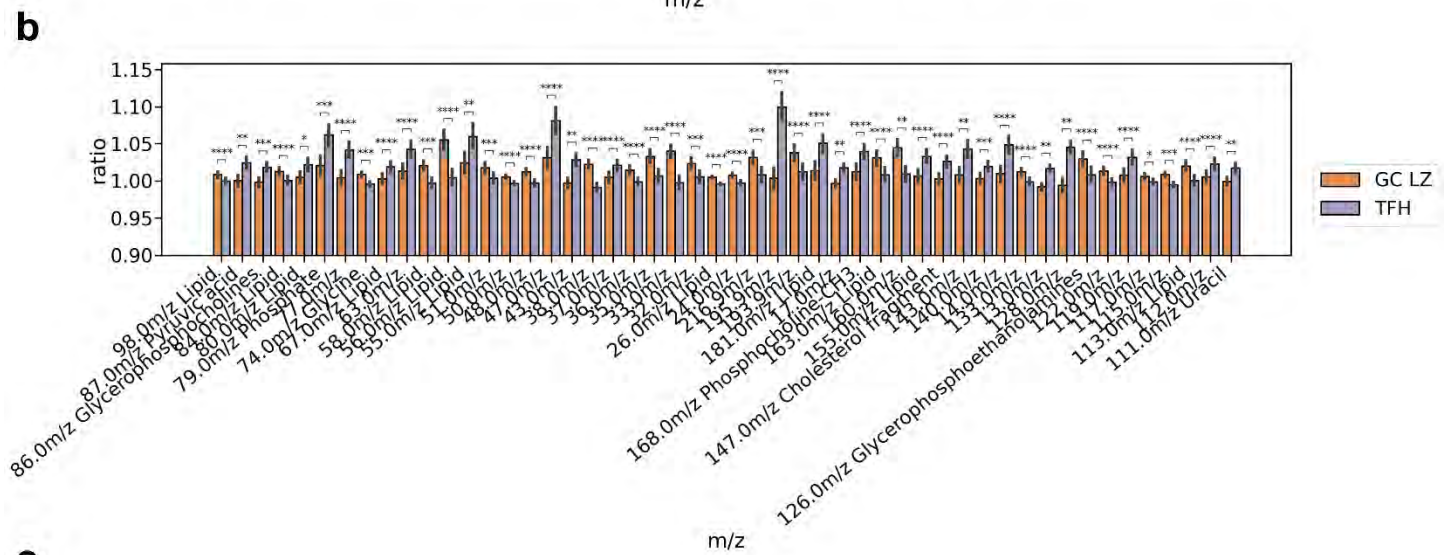
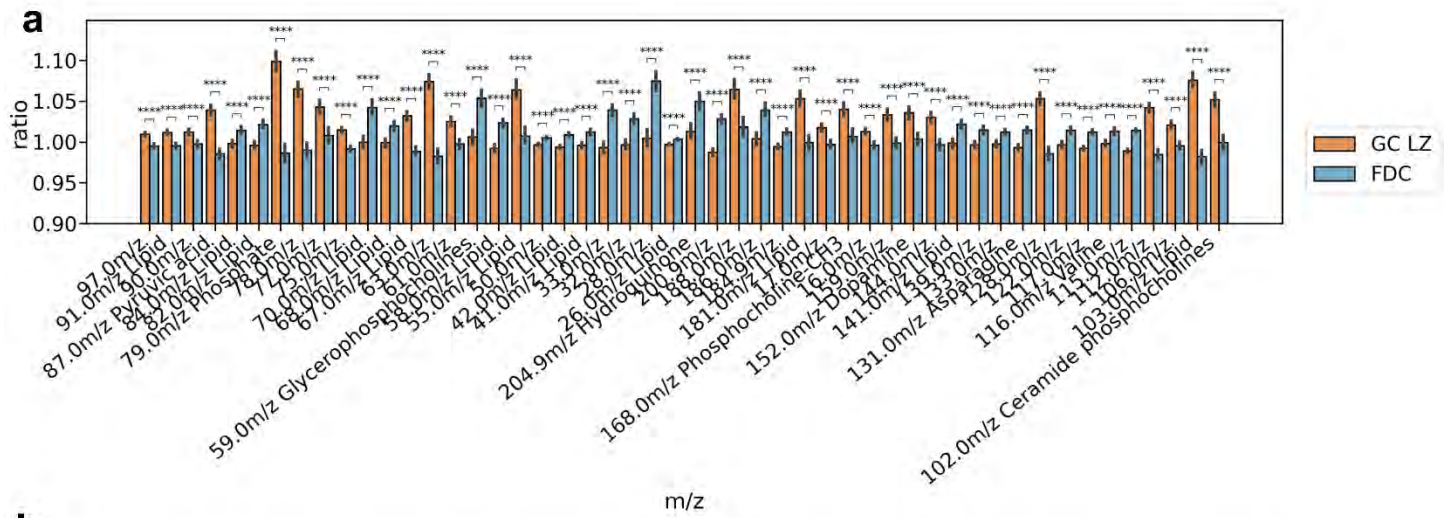


Tonsil Donor E



Supplementary Fig. 29. Single-cell joint protein-metabolite phenotyping in tonsil follicles (n=31156 cells).

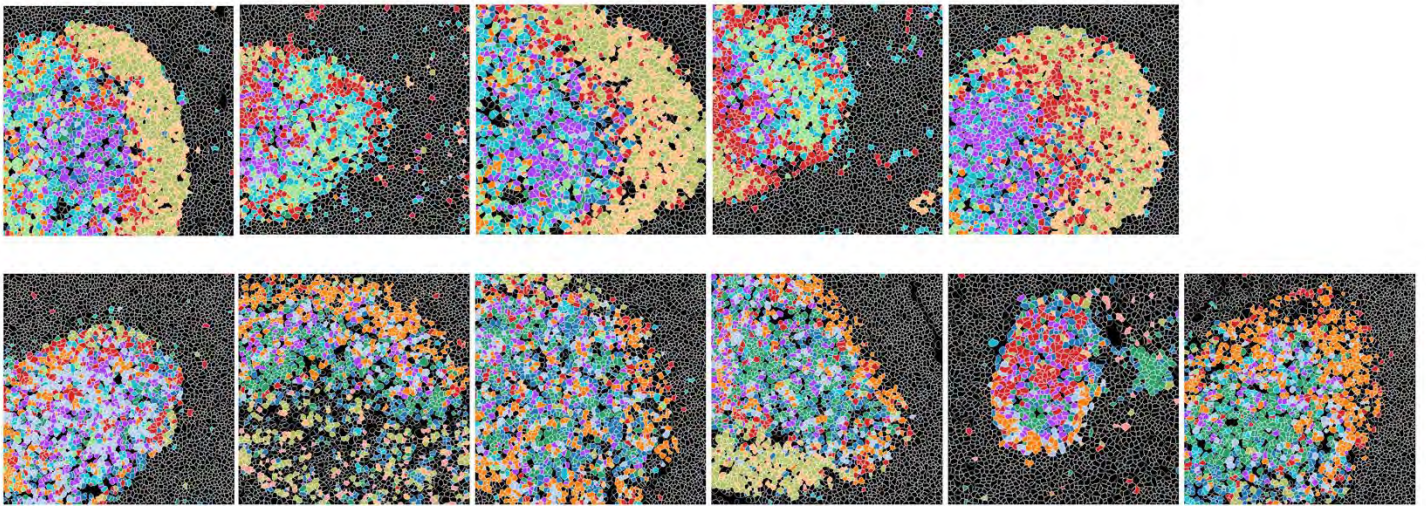
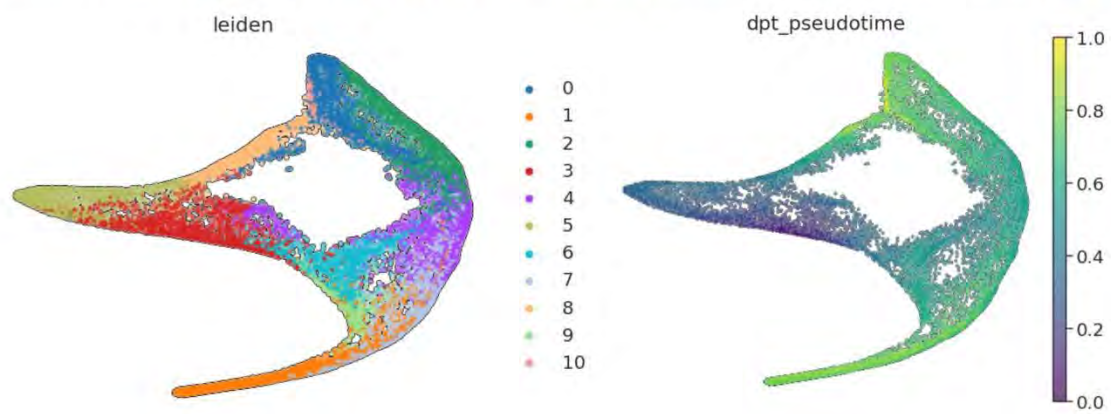
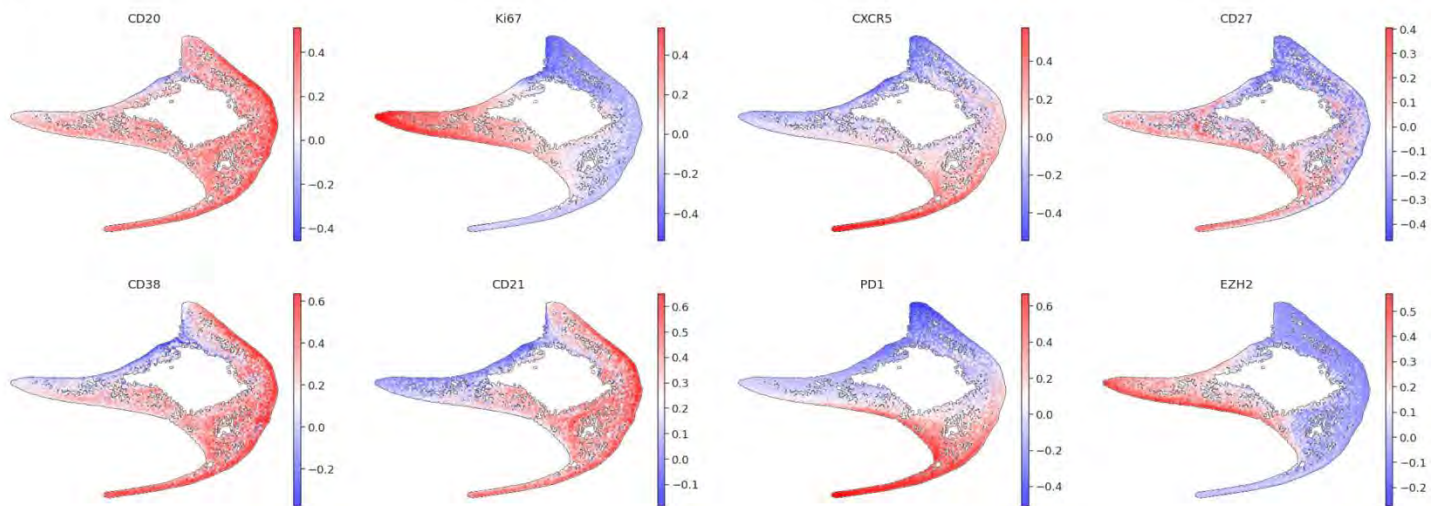
- a** VAE single cell protein-metabolite joint embedding. Left: architecture for single-cell protein-metabolite joint embedding. Right: joint protein-metabolites single-cell phenotype.
- b** Spatial projection of clusters obtained from **a** human tonsil tissues for all imaged follicle regions.
- c** Density of unsupervised cluster in defined cell neighborhoods.
- d** The density of each cell neighborhood across the follicle imaging regions.
- e** Spatial projection of cell neighborhood obtained from **c** and **d** in human tonsil tissues for all imaged regions of interest.



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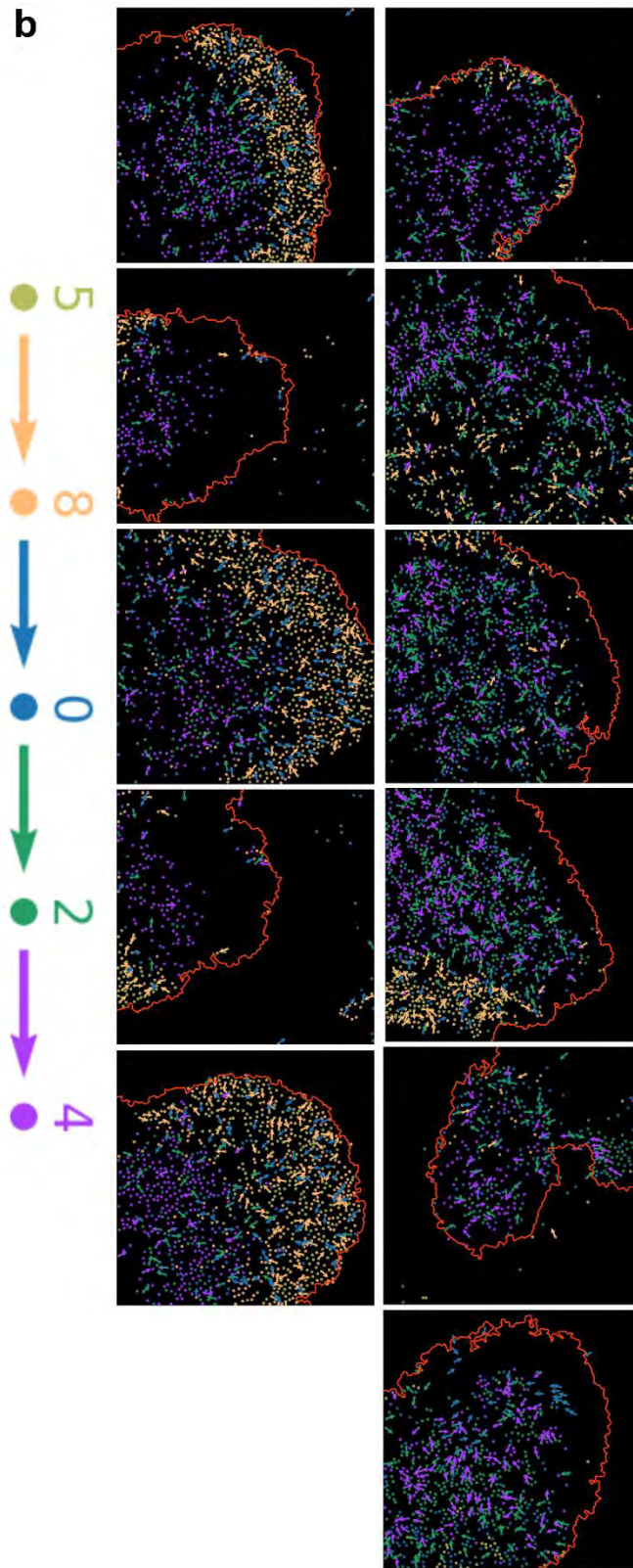
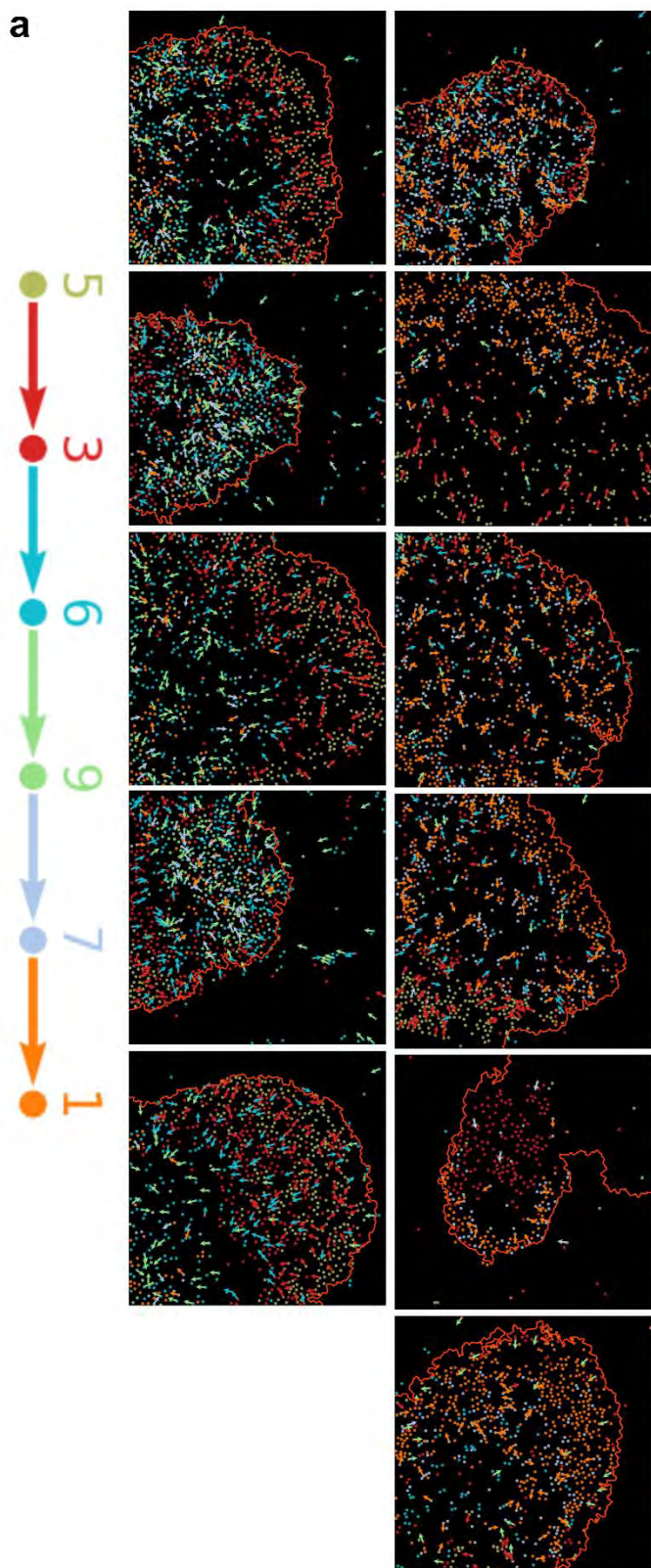
231

Supplementary Fig. 30. Single-cell local metabolite competition in tonsil tissues. Local competition of metabolites between B cells and FDCs (**a**) (n=4371 cells), B cells and TFHs (**b**) (n=2807 cells), B cells in LZ with DZ (**c**) (n=1870 cells). Mann-Whitney-Wilcoxon test was two-sided with Bonferroni correction (ns: $0.05 < p$, ****: $p \leq 0.0001$).

a**b****c**

Supplementary Fig. 31. B-cell trajectory inside germinal centers (n=31156 cells).

- a** Spatial projection of selected B-cell clusters from protein profiles in human tonsil tissues for all imaged follicle regions.
- b** Pseudotime analysis of B-cells. Left: t-SNE embedding and unsupervised clustering of B-cells. Right: Pseudotime analysis.
- c** Projection of single-cell marker intensity on t-SNE map.

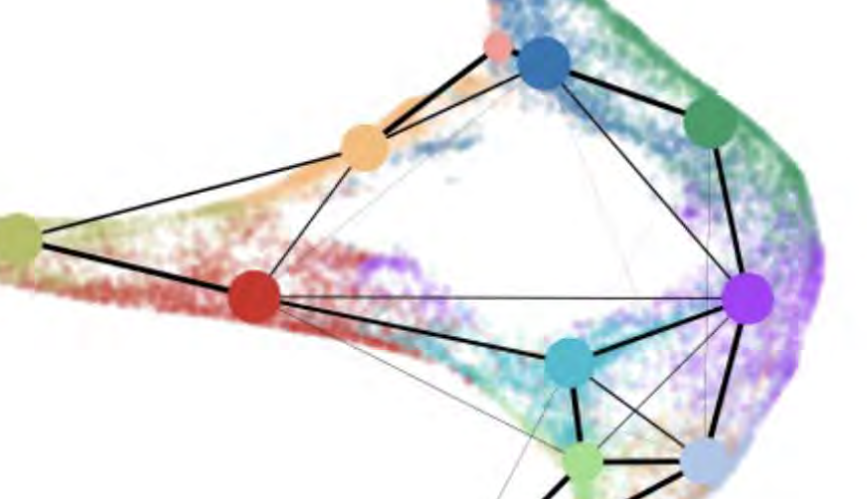


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Supplementary Fig. 32. Spatial projection of B-cell trajectory inside germinal centers.

- a** Spatial projection of selected B-cell pseudotime differentiation analysis from the germinal center dark zone to the germinal center light zone. Left: Arrows showing the trajectory along each cell cluster. Right: Spatial projection of single cells. Schematics of arrows and colors were created with Biorender.com.
- b** Spatial projection of selected B-cell pseudotime differentiation analysis from germinal center dark zone to activated B-cells. Left: Arrows showing the trajectory along each cell cluster. Right: Spatial projection of single cells. Schematics of arrows and colors were created with Biorender.com.



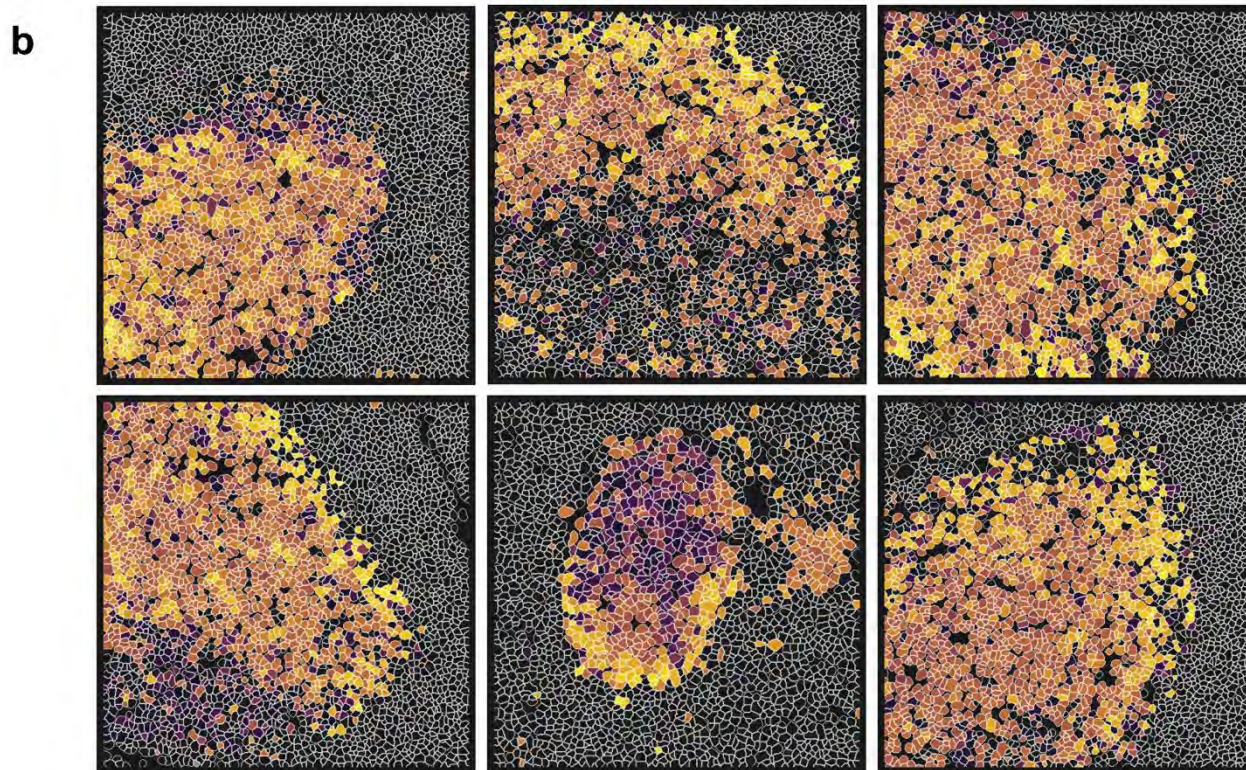
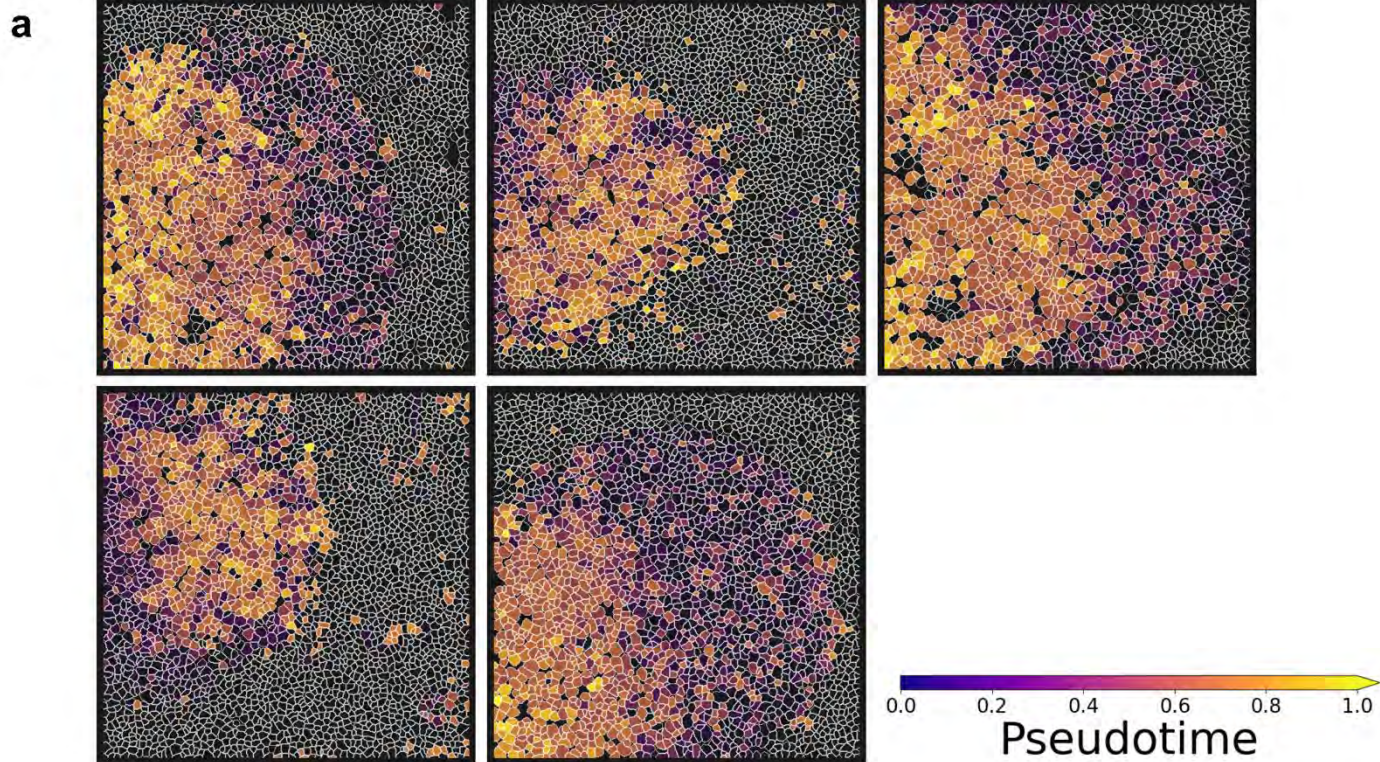
cluster

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Supplementary Fig. 33. Projection of B-cell trajectory on t-SNE.

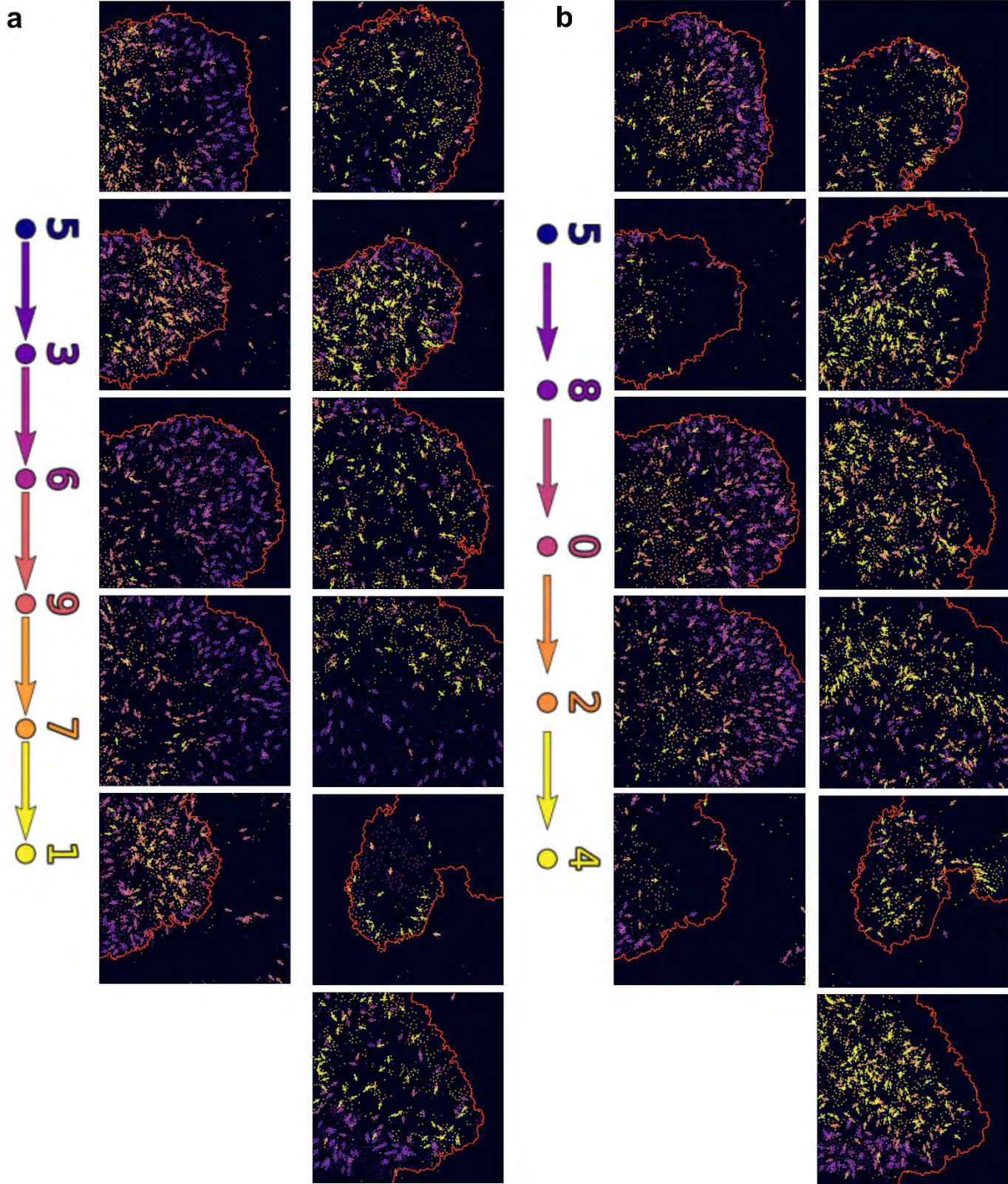
- a** Single-cell t-SNE embedding showing the trajectory of B-cells inside germinal center regions. Arrows showing the trajectory along each cell cluster.
- b** The graph-directed plot of B-cell embedding on t-SNE.



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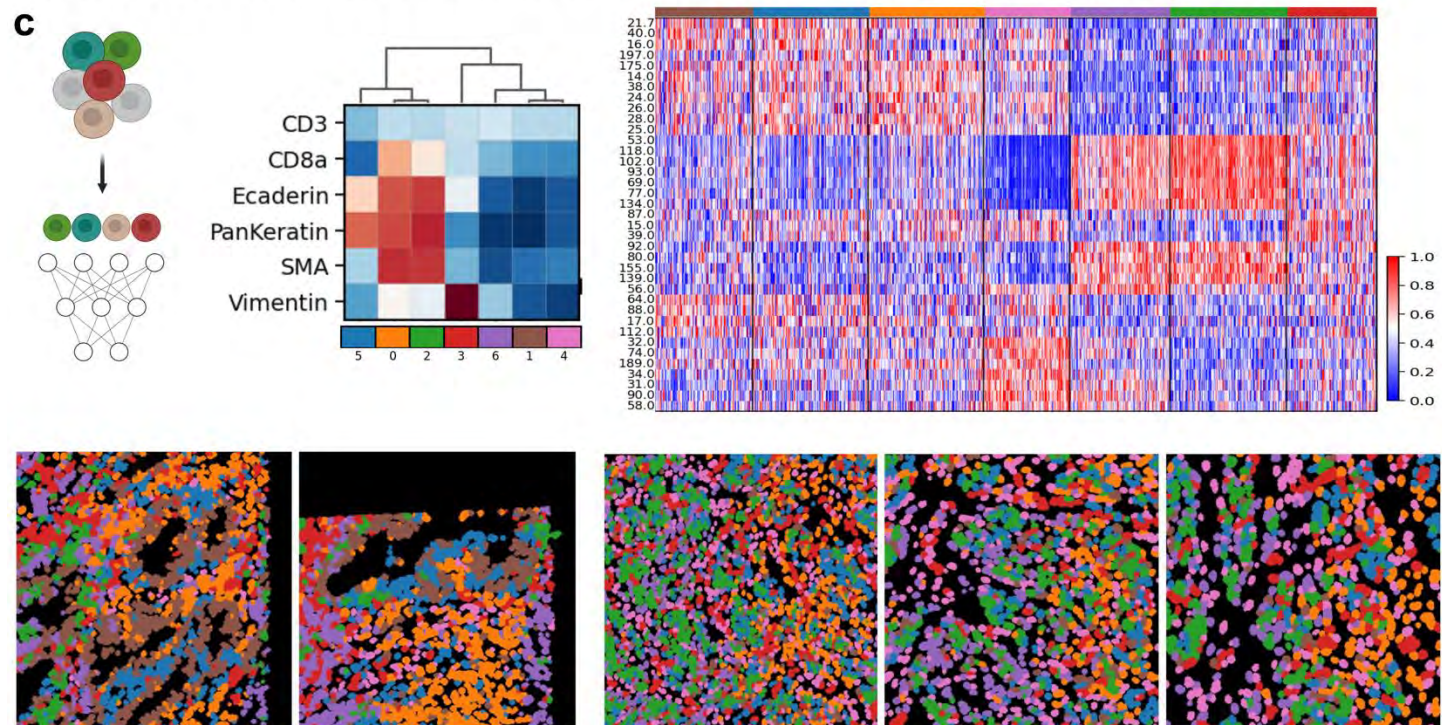
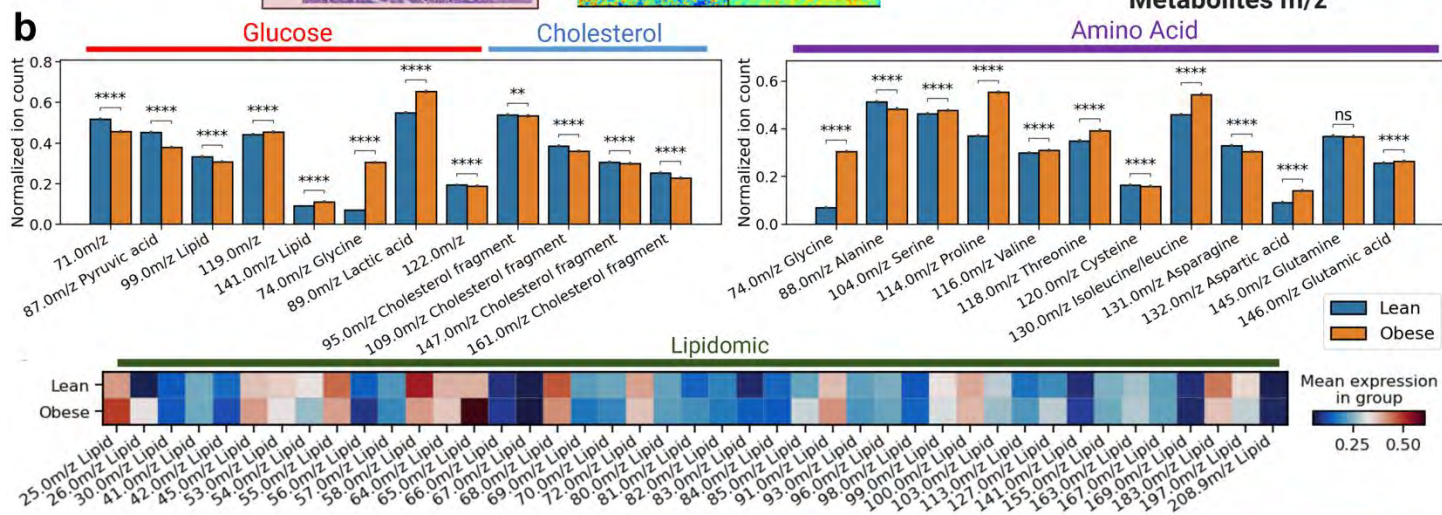
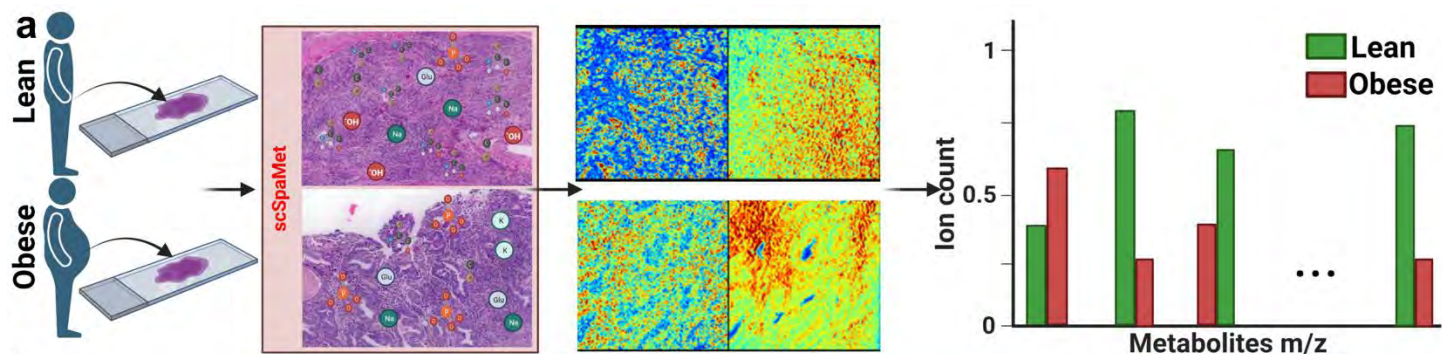
264

Supplementary Fig. 34. Spatial projection of B-cell trajectory inside germinal centers. Spatial projection of single-cell with corresponding pseudotime value from pseudotime analysis in tonsil donor A **a** and tonsil donor B **b**.



Supplementary Fig. 35. Spatial projection of B-cell trajectory inside germinal centers.

- a** Spatial projection of selected B-cell pseudotime differentiation analysis from the germinal center dark zone to the germinal center light zone. Left: Arrows showing the trajectory along each cell cluster with corresponding pseudotime value. Right: Spatial projection of single cells with corresponding pseudotime value. Schematics of arrows and colors were created with Biorender.com.
- b** Spatial projection of selected B-cell pseudotime differentiation analysis from germinal center dark zone to activated B-cells. Left: Arrows showing the trajectory along each cell cluster with corresponding pseudotime value. Right: Spatial projection of single cells with corresponding pseudotime value. Schematics of arrows and colors were created with Biorender.com.

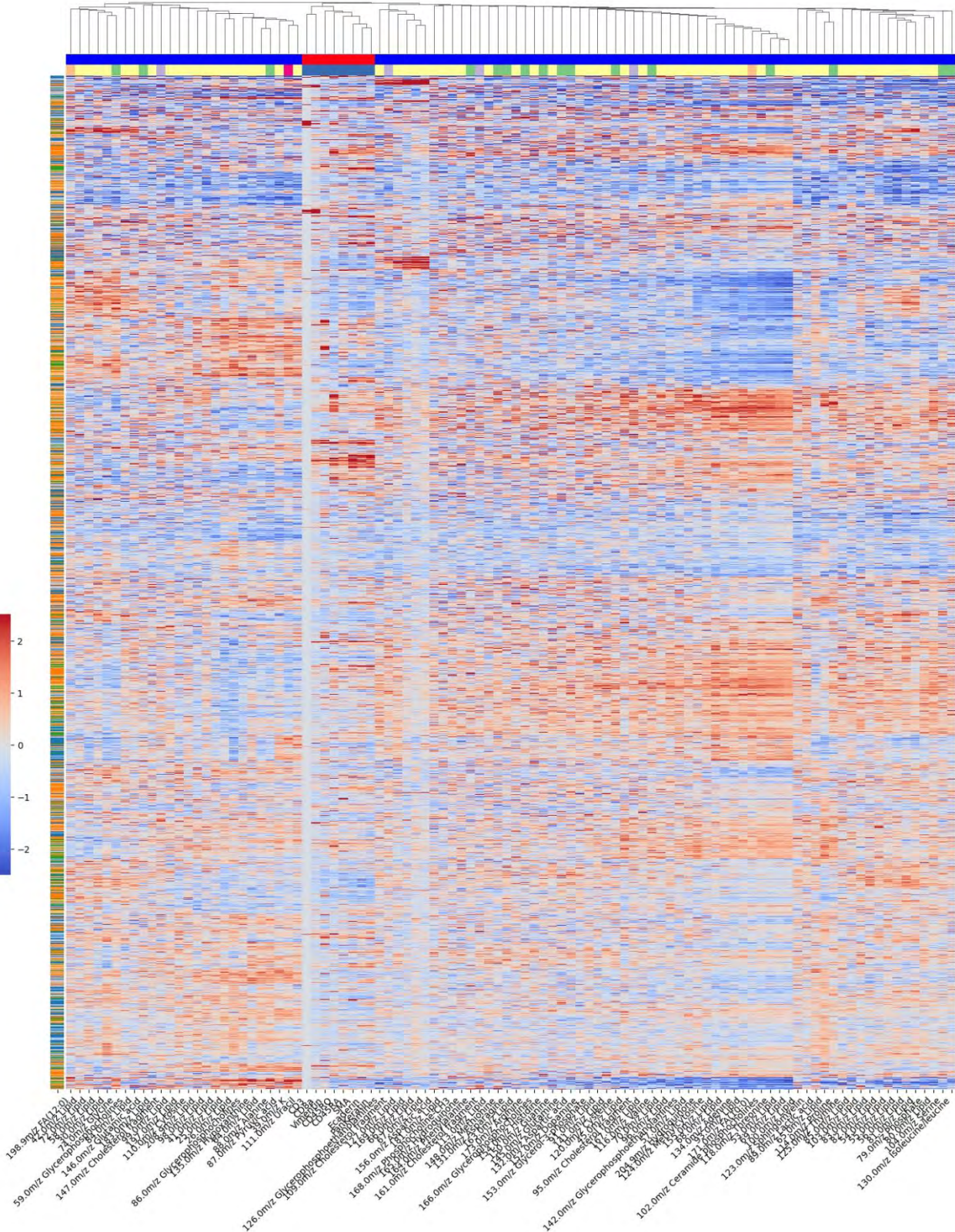
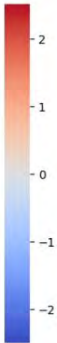


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Supplementary Fig. 36. ScSpaMet quantifies metabolite differences of lean and obese patients in human endometrium samples.

- a** Representative schematic showing the comparison of metabolite expression in lean and obese samples from human endometrium. Created with Biorender.com.
- b** Comparison of single cell metabolic expression level for identified metabolite channels between lean and obese samples (n=8215 cells). Left: metabolite channels related to Glucose pathway and Cholesterol fragments. Right: Metabolite channels related to amino acid fragments. Mann-Whitney-Wilcoxon test was two-sided with Bonferroni correction (ns: $0.05 < p$, ****: $p \leq 0.0001$).
- c** Metabolite channels related to identified lipid channels fragmentation.

ENDOMETRIUM

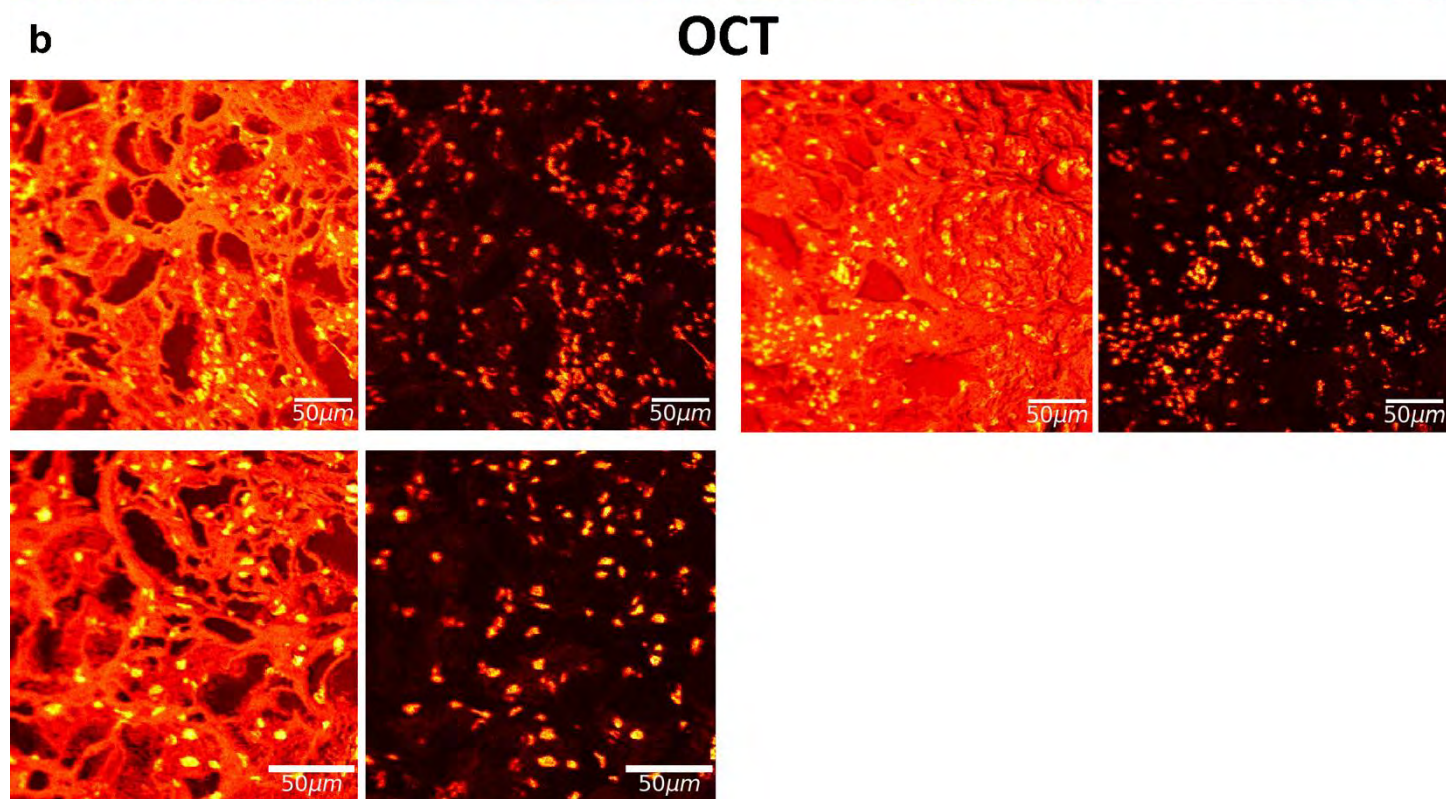
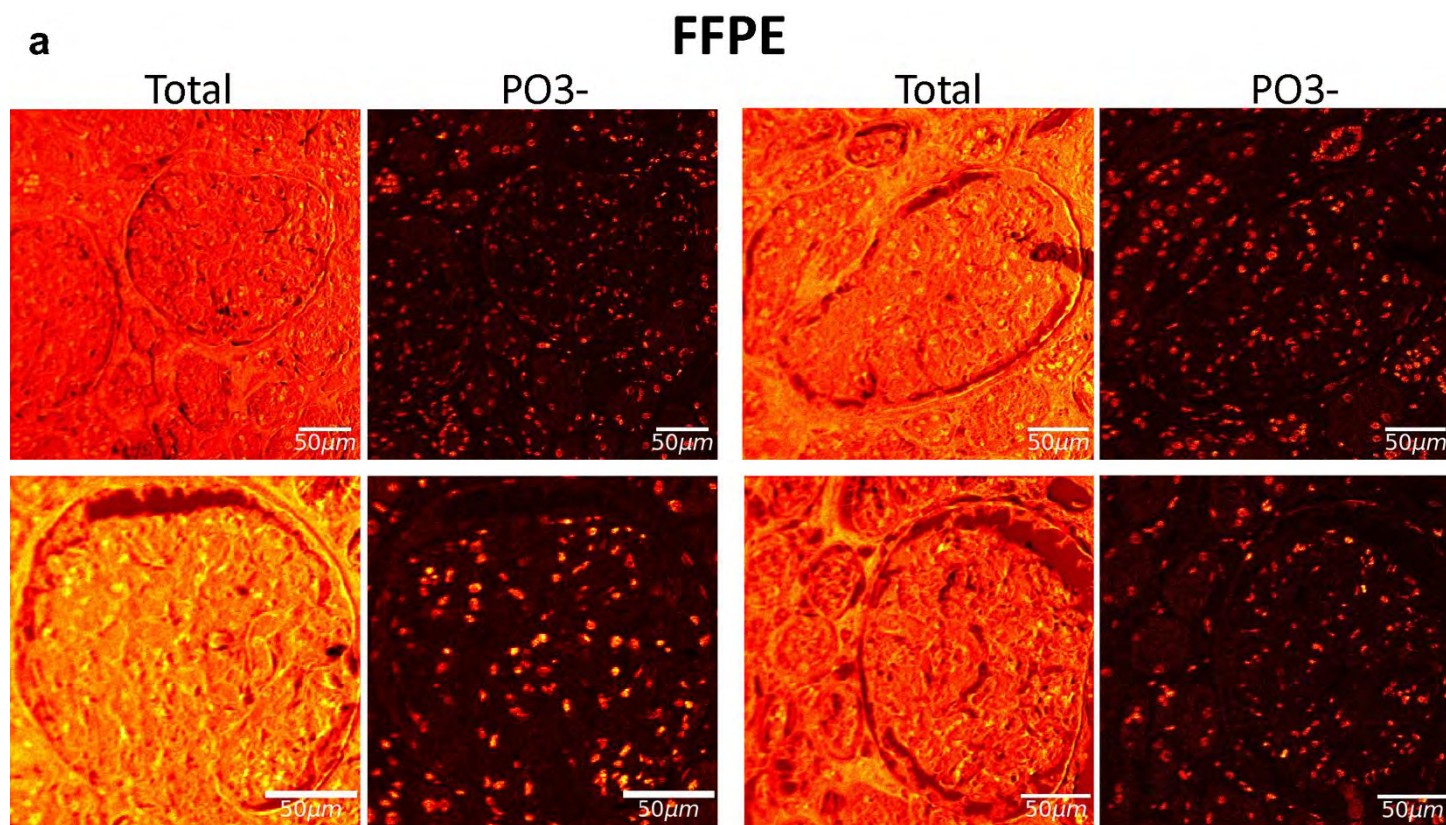


295 **Supplementary Fig. 37.** Single-cell metabolite and protein profile in endometrium tissues (n=8215 cells). Cluster
296 map showing the cell-level metabolite and protein profile in all endometrium tissues. Colorbar corresponds to the
297 mean intensity value at the single cell level.

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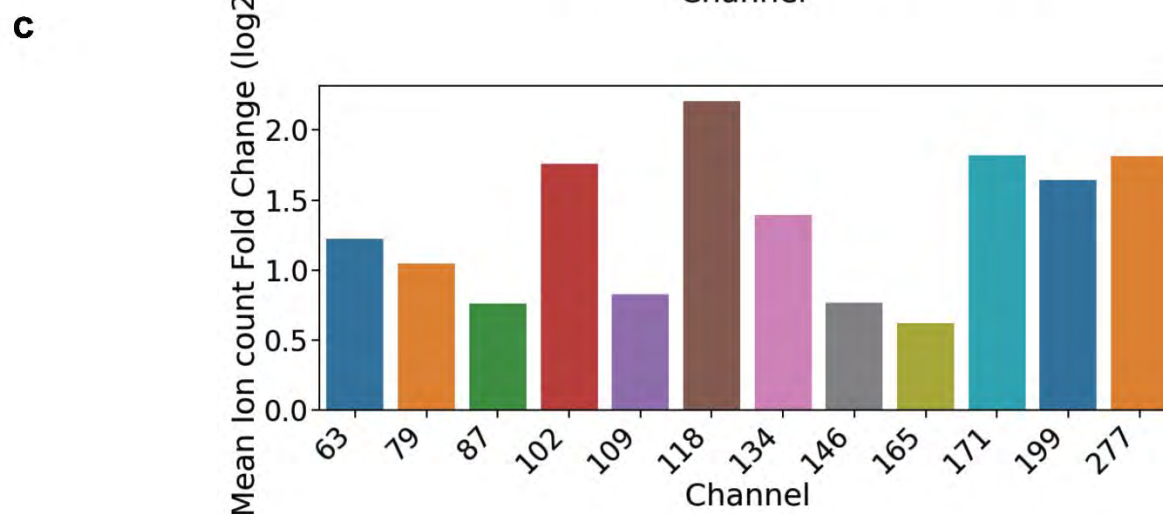
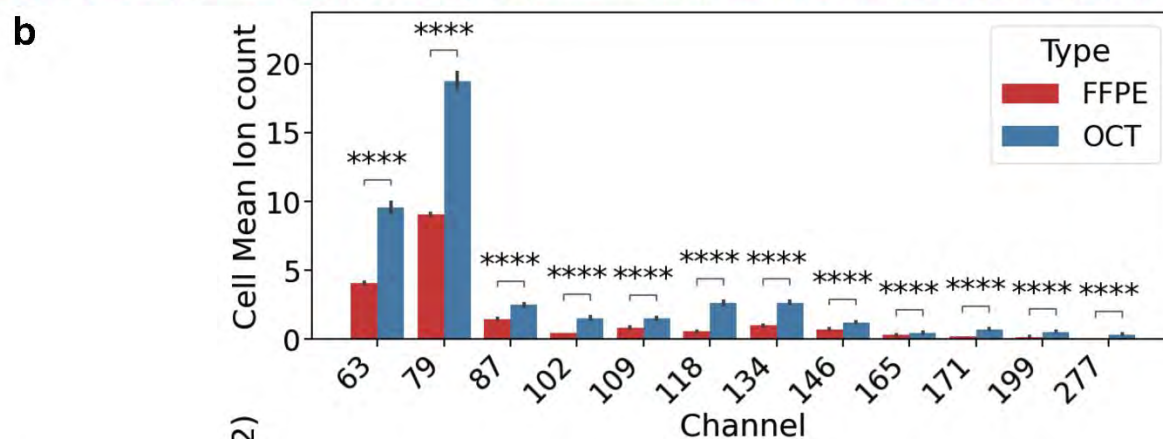
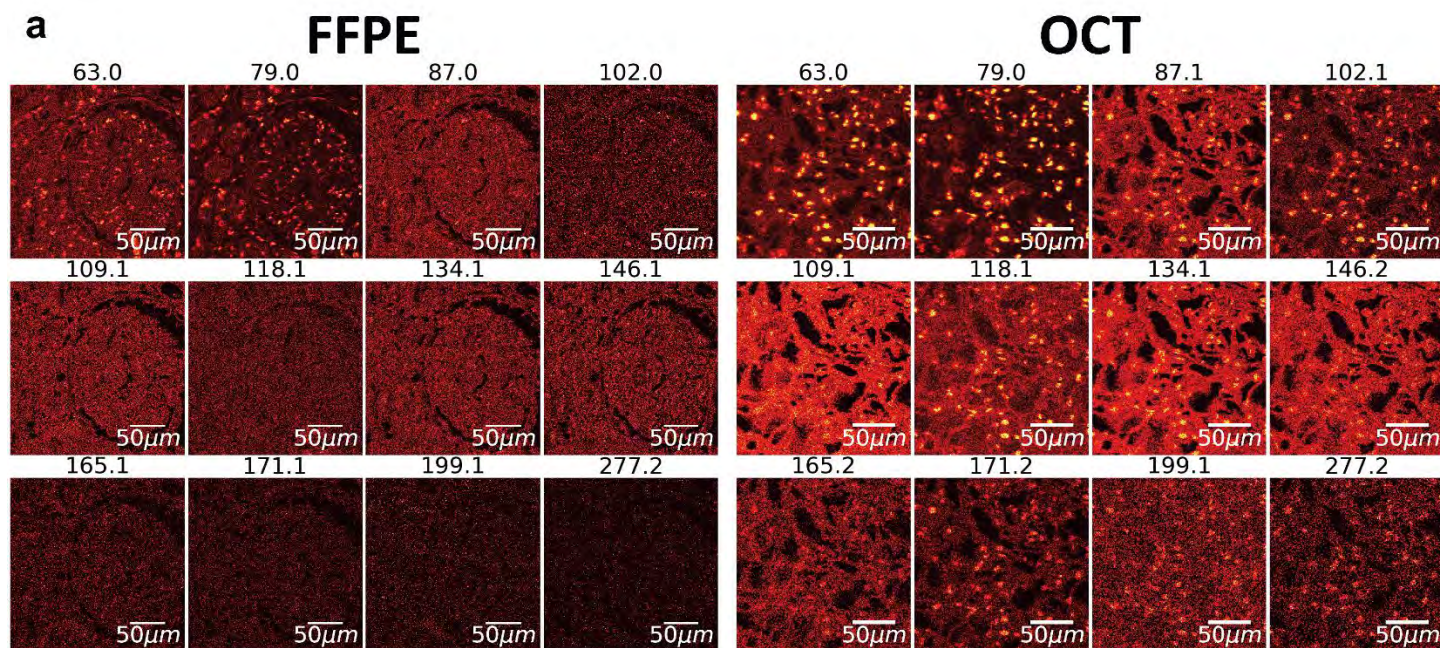
303 **Supplementary Fig. 38.** Summary of findings from joint proteomic and metabolomic analysis from our data in
304 **a** lung cancer tissues and **b** tonsil tissues at the single cell level. Created with Biorender.com

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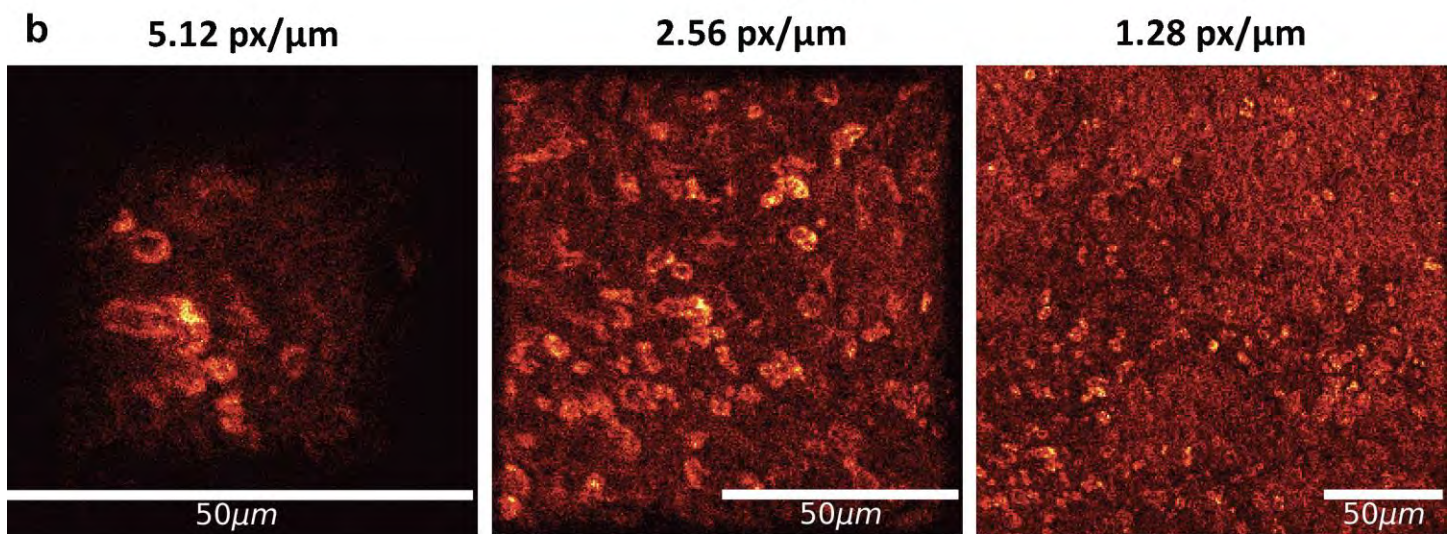
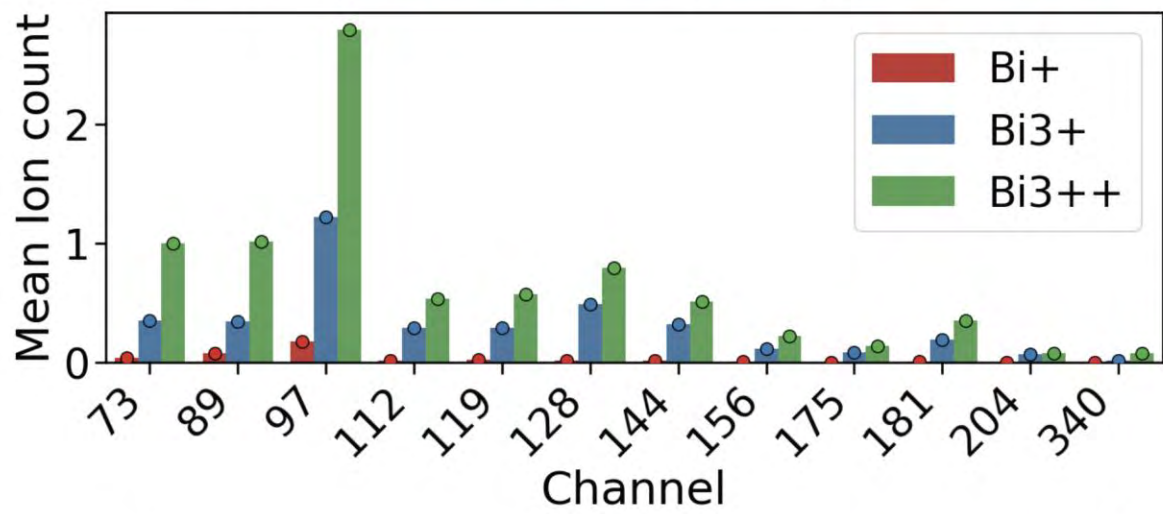
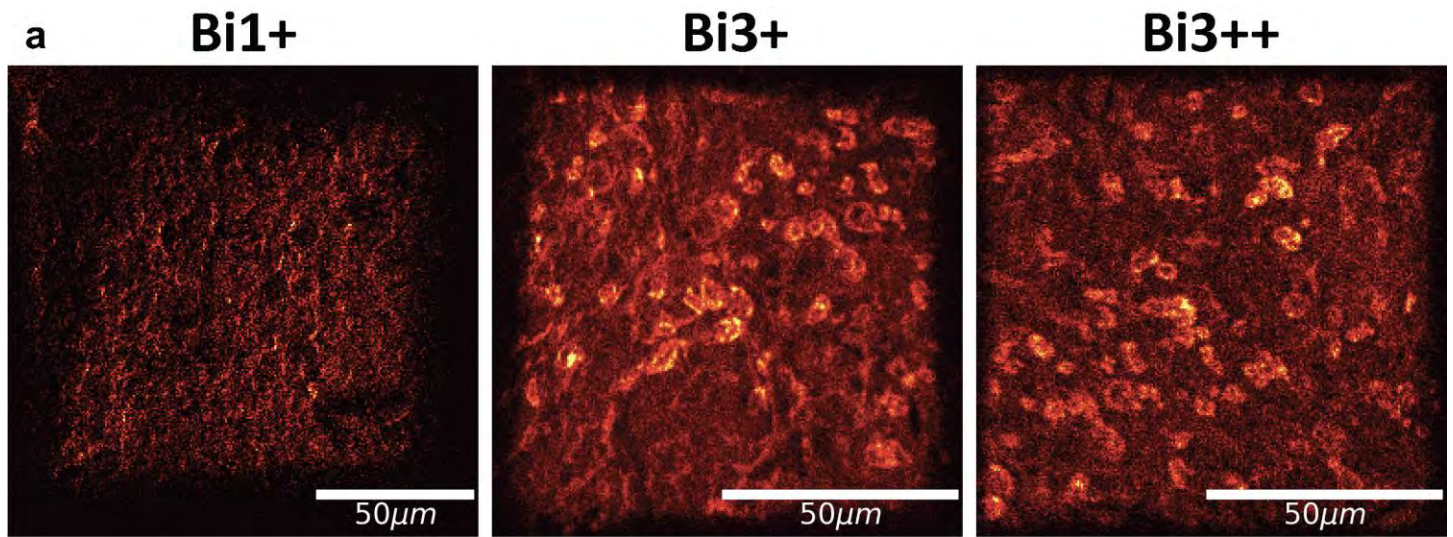


309 **Supplementary Fig. 39.** Comparison of TOF–SIMS imaging for **a** FFPE and **b** OCT kidney tissue samples. Scale
310 bar 50 μm .

311



314 **Supplementary Fig. 40.** Comparison of TOF–SIMS imaging for FFPE (n = 41 cells) and OCT kidney tissue (n
315 = 35 cells) samples in selected channels **a**, mean ion count per cell **b**, and mean ion count fold change **c**.
316 Mann-Whitney-Wilcoxon test was two-sided with Bonferroni correction (****: $p \leq 0.0001$). Scale bar 50 μm .
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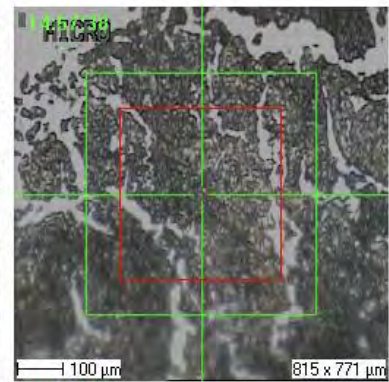
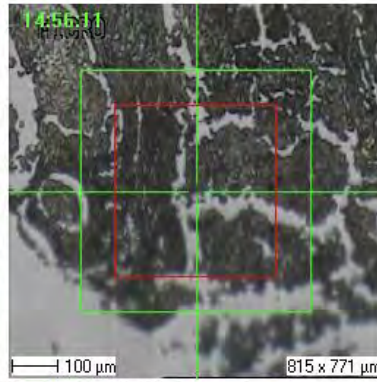
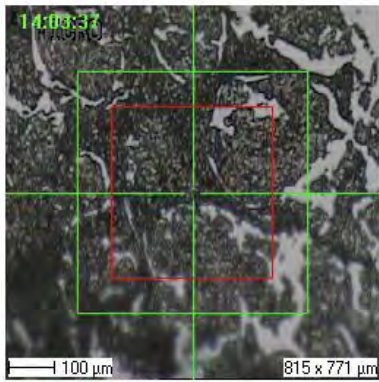


321 **Supplementary Fig. 41.** Comparison of TOF-SIMS imaging setting on lung cancer tissues. **a** Comparison of the
322 three TOF-SIMS bismuth liquid metal ion gun modes (Bi^+ , Bi^{3+} , Bi^{3++}) for extracting single-cell features. Bar
323 plot showing the mean ion count per pixel in selected channels. **b** Comparison of imaging regions size (50 μm ,
324 100 μm , 200 μm) at 256x256 pixels density. Scale bar 50 μm .

325

LUNG CANCER

B5



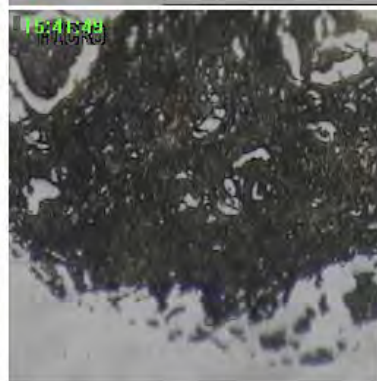
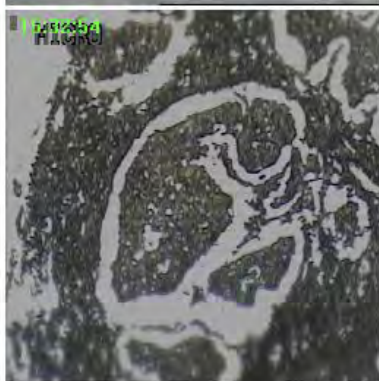
D4



E6



F4



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327

328 **Supplementary Fig. 42.** Bright field imaging from TOF-SIMS showing tissue regions in human lung cancer
329 tissues.

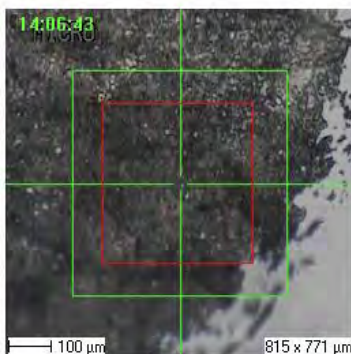
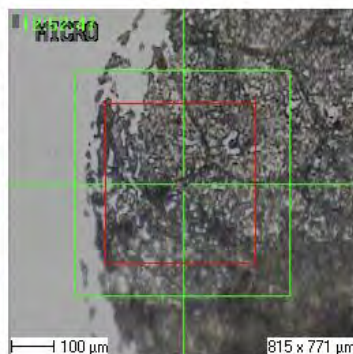
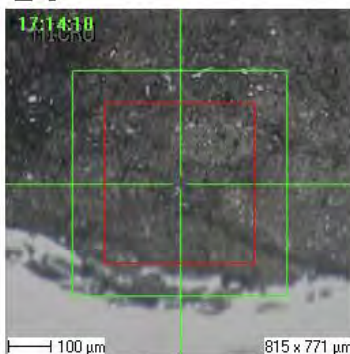
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C6

LUNG CANCER



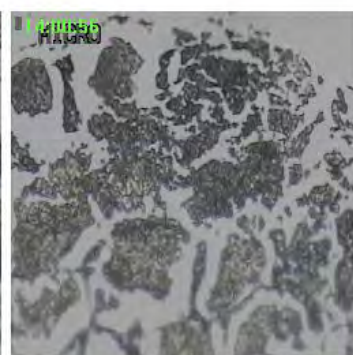
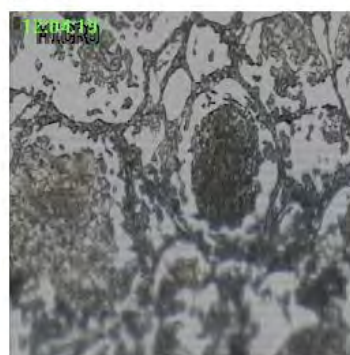
E4



F7



D2



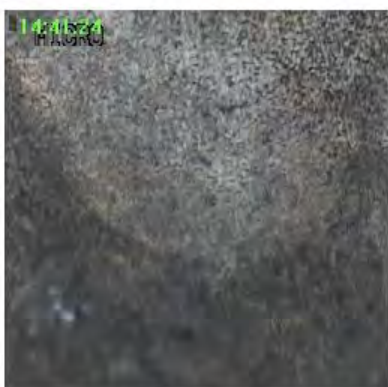
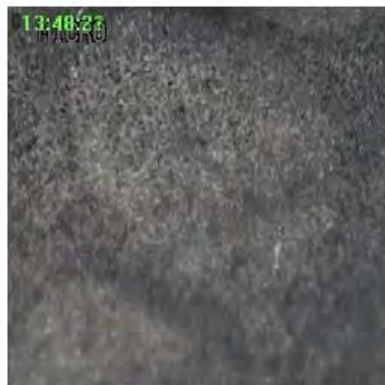
333 **Supplementary Fig. 43.** Bright field imaging from TOF-SIMS showing tissue regions in human lung cancer
334 tissues.

335

TONSIL DONOR A



TONSIL DONOR E



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338 **Supplementary Fig. 44.** Bright field imaging from TOF-SIMS showing tissue regions in human tonsil tissues.

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Endometrium Obese



Endometrium Lean



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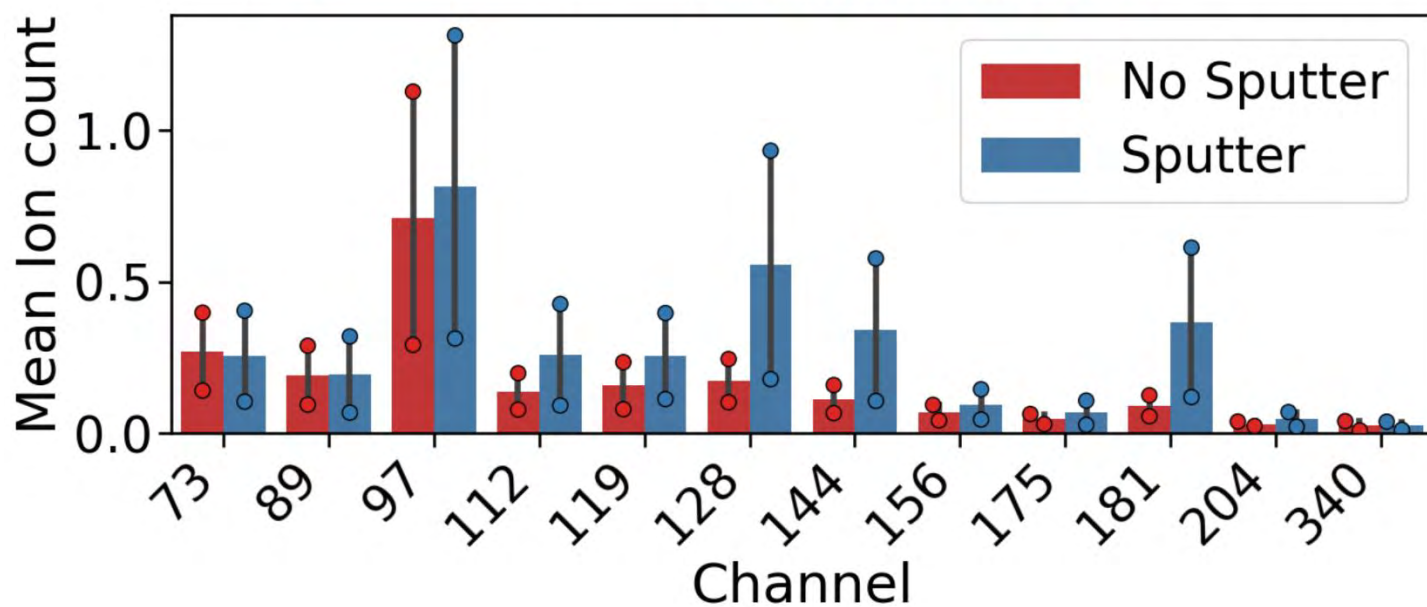
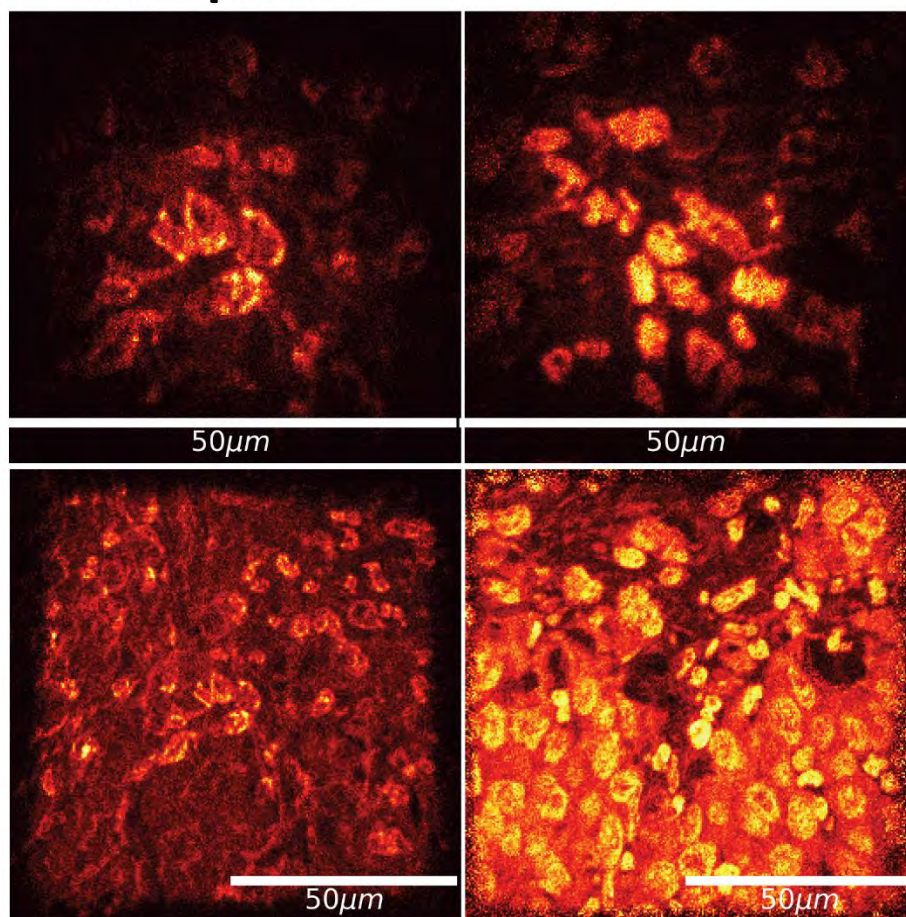
351 **Supplementary Fig. 45.** Bright field imaging from TOF-SIMS showing tissue regions in human endometrium
352 tissues.

353

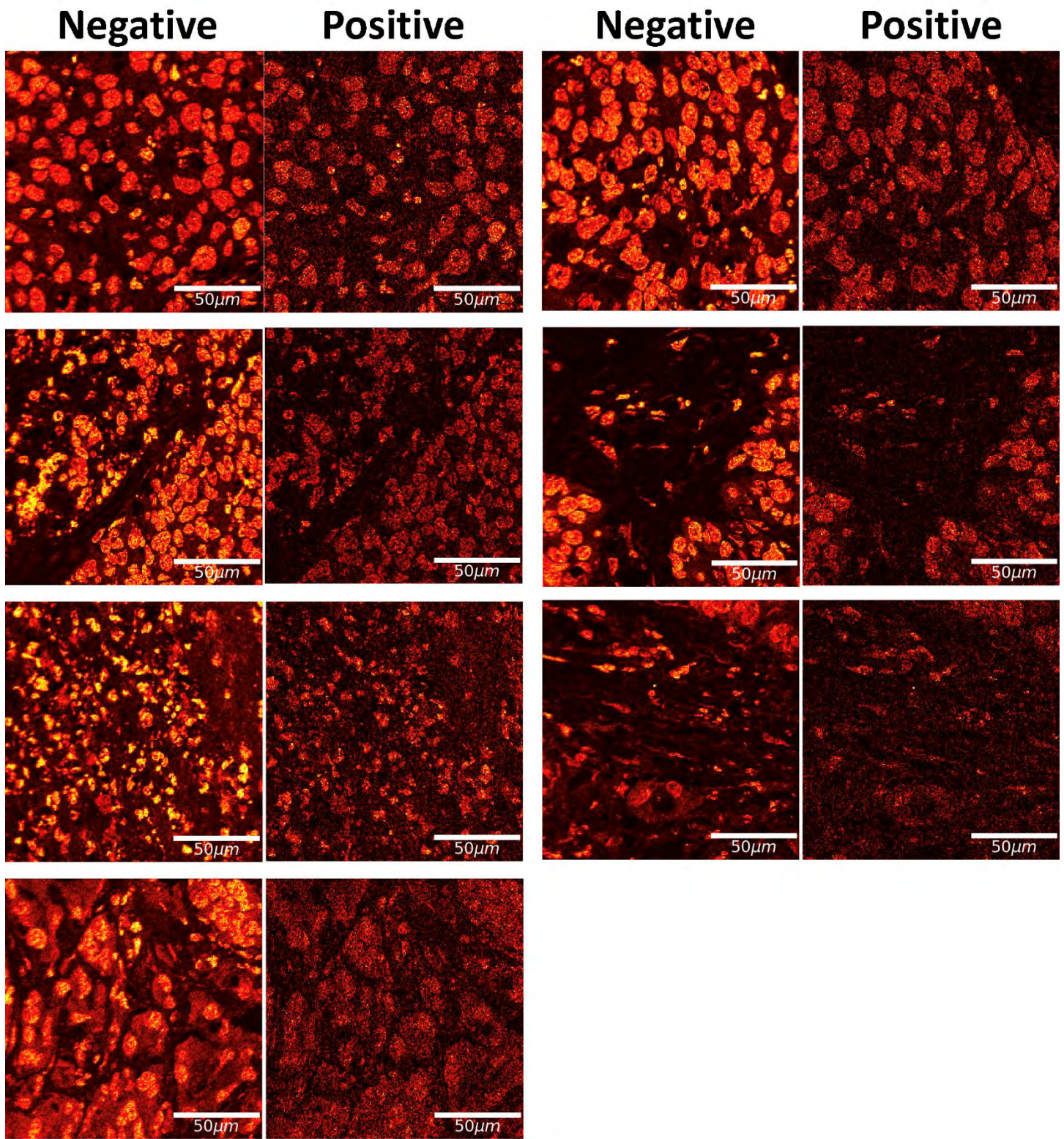
354

No Sputter

Sputter



357 **Supplementary Fig. 46.** Comparison of TOF–SIMS imaging with and without sputtering. Bar plot showing the
358 mean ion count when using a sputtering source for depth profiling (n = 2 samples). Scale bar 50 μm .
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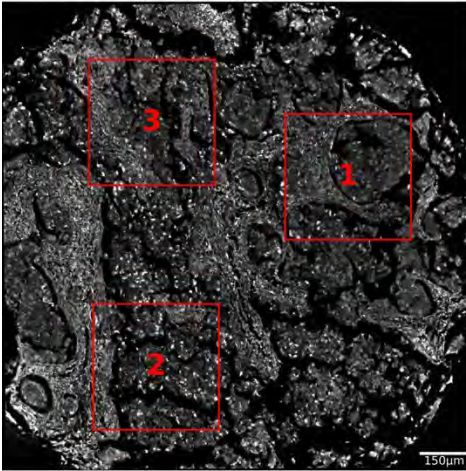
362

363 **Supplementary Fig. 47.** Comparison of TOF–SIMS imaging in positive and negative mode. Scale bar 50 μm .

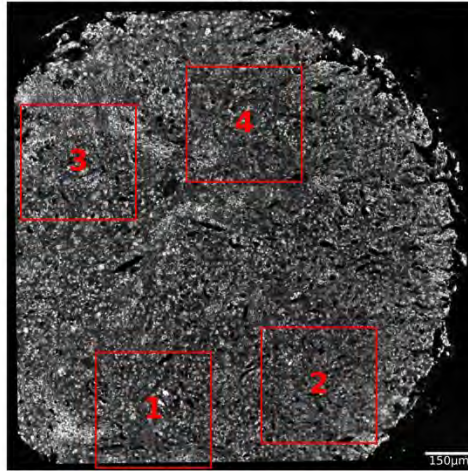
364

LUNG CANCER

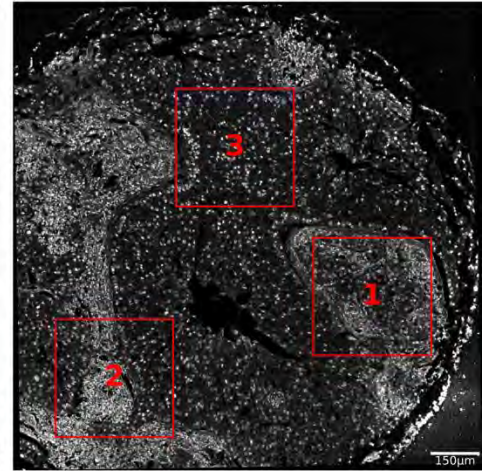
B5



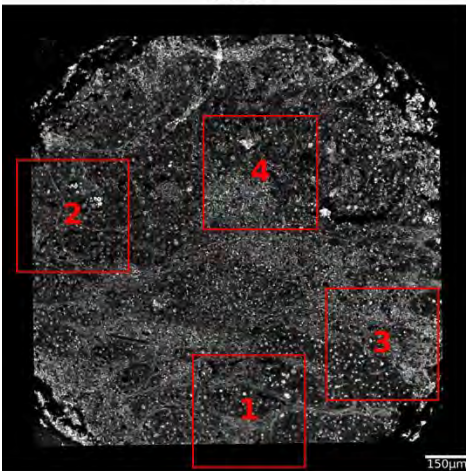
C6



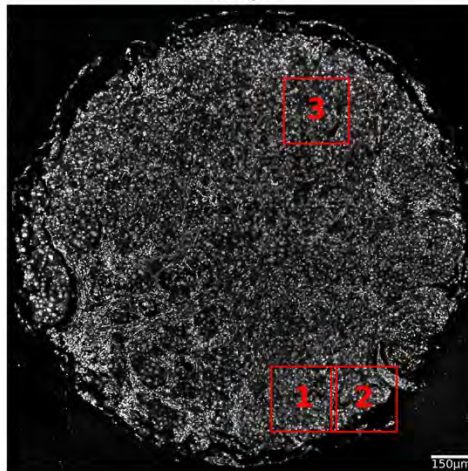
D4



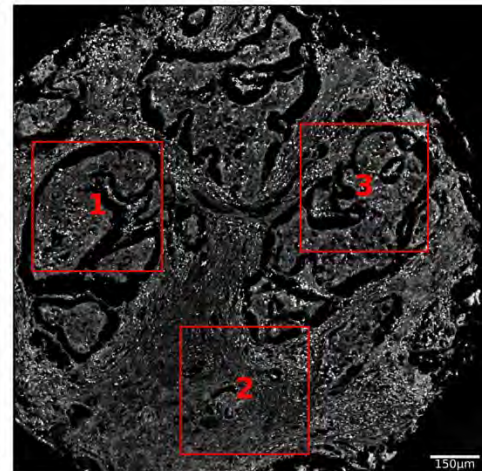
E4



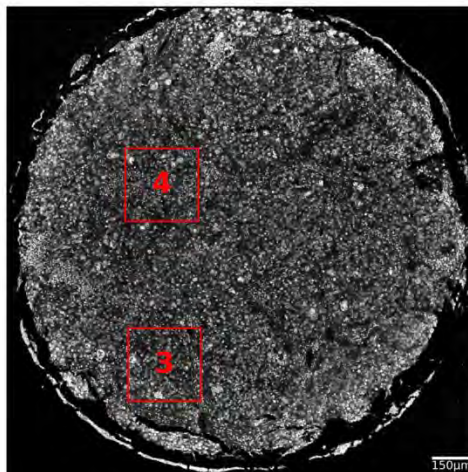
E6



F4



F7



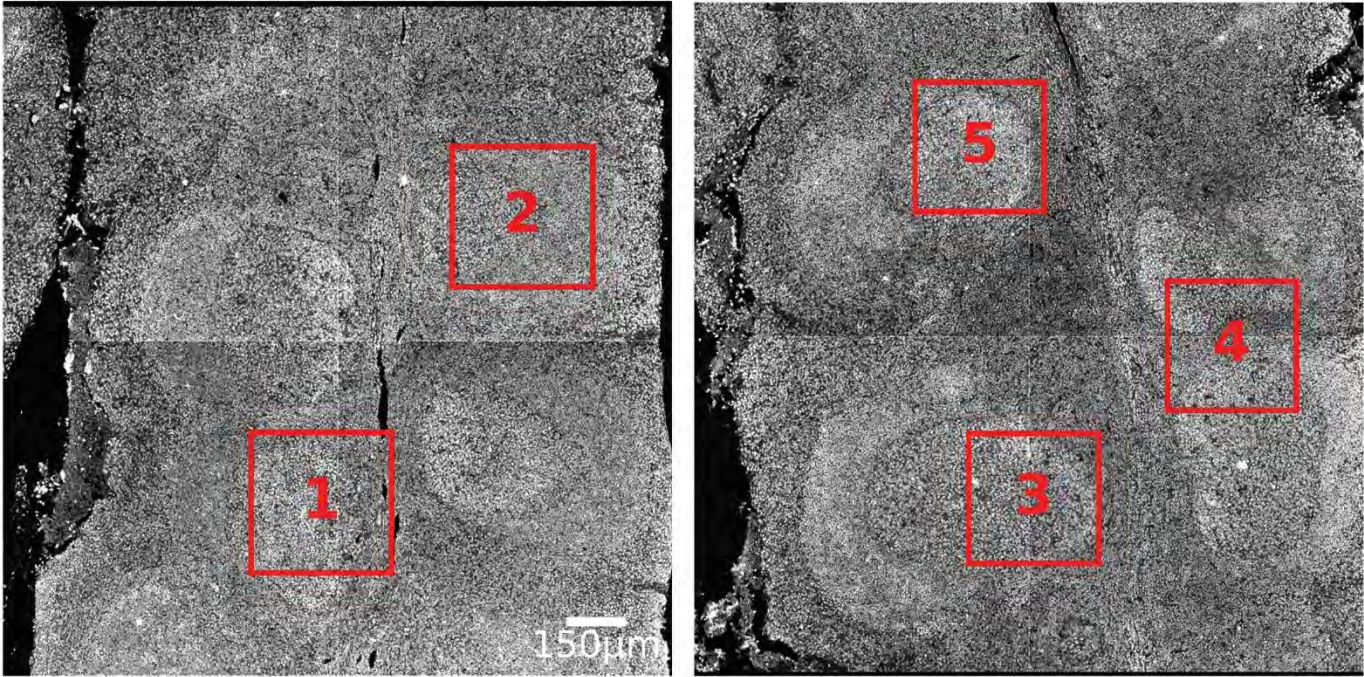
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366

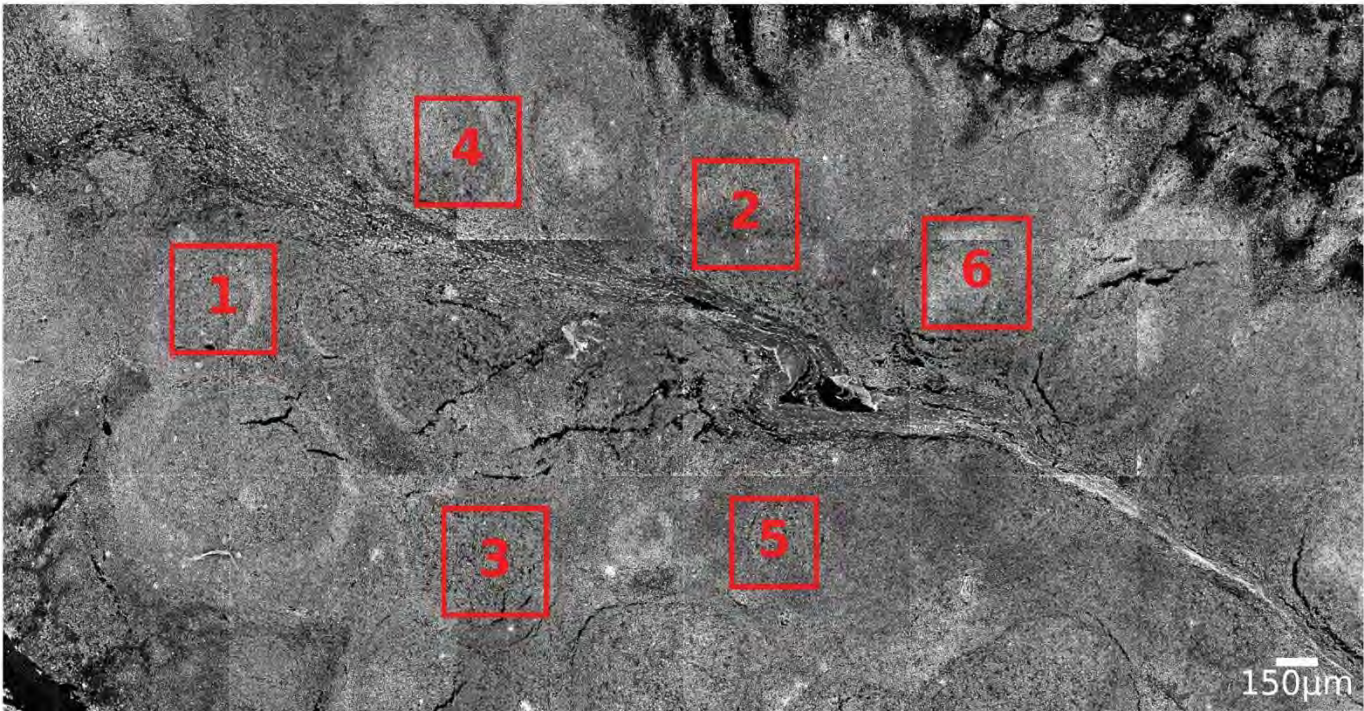
367 **Supplementary Fig. 48.** TOF–SIMS imaging regions highlighted in the IMC Histone H3 marker images in
368 human lung cancer tissues. Scale bar 150 μm .

369

TONSIL DONOR A



TONSIL DONOR E

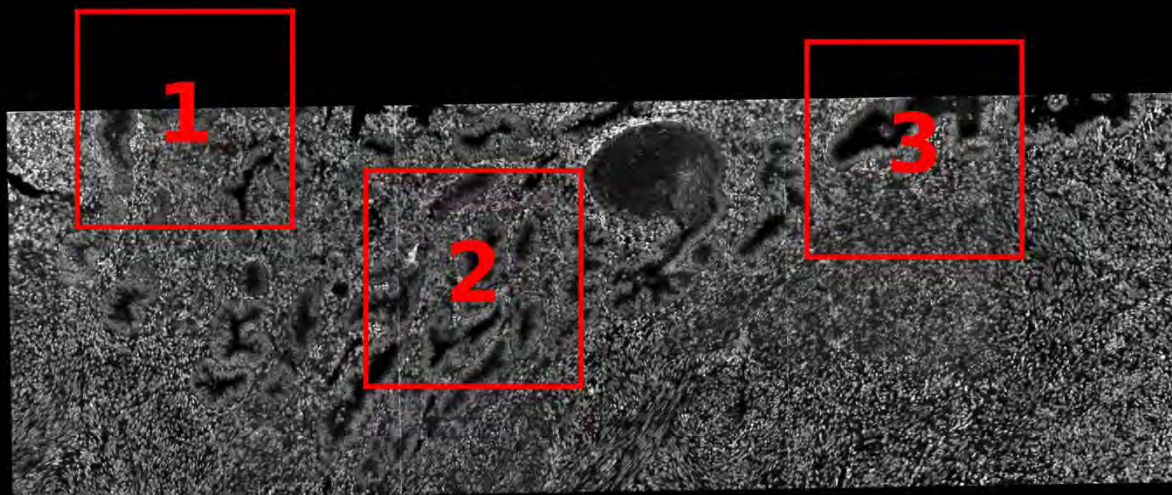


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373 **Supplementary Fig. 49.** TOF–SIMS imaging regions showed in the IMC Intercalator marker images in human
374 tonsil tissues. Scale bar 150 μm .

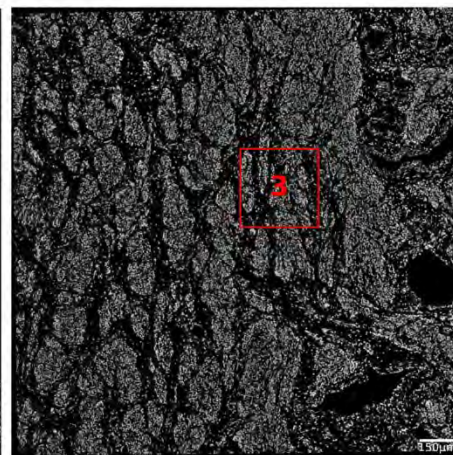
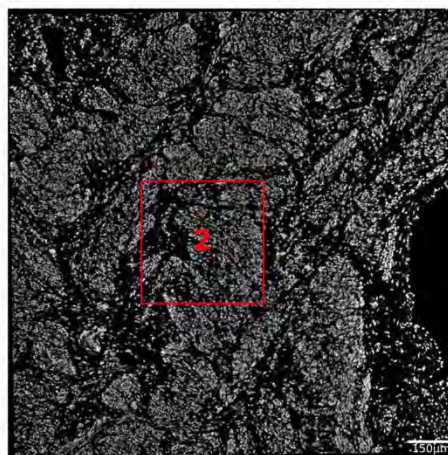
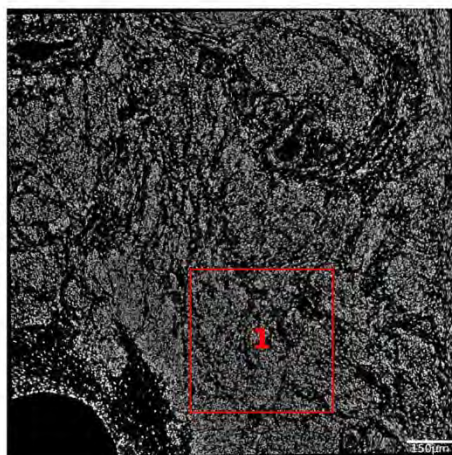
375

Obese



150µm

Lean



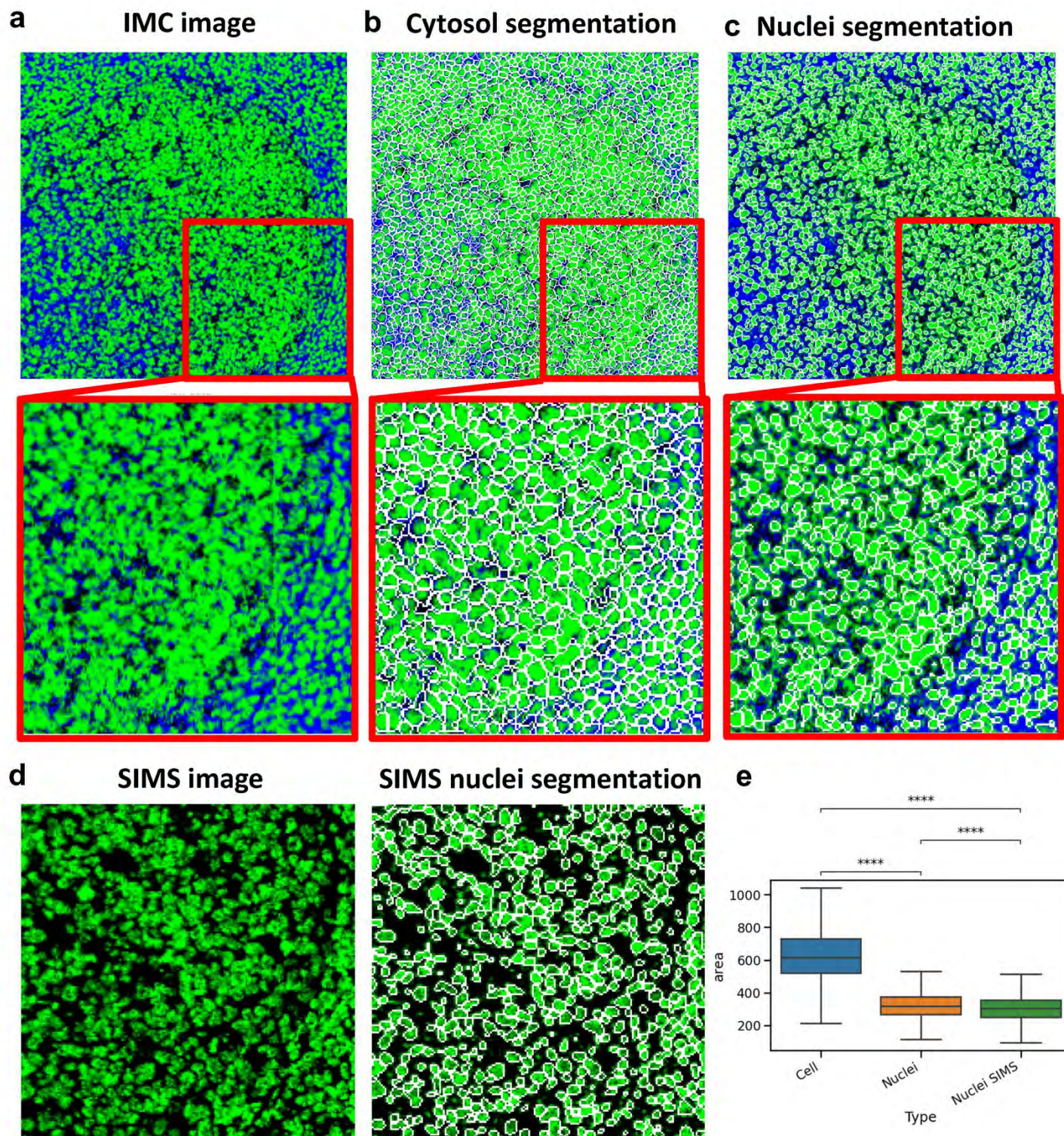
376

377

378

379 **Supplementary Fig. 50.** TOF–SIMS imaging regions showed in the IMC Intercalator marker images in human
380 endometrium tissues. Scale bar 150 μm .

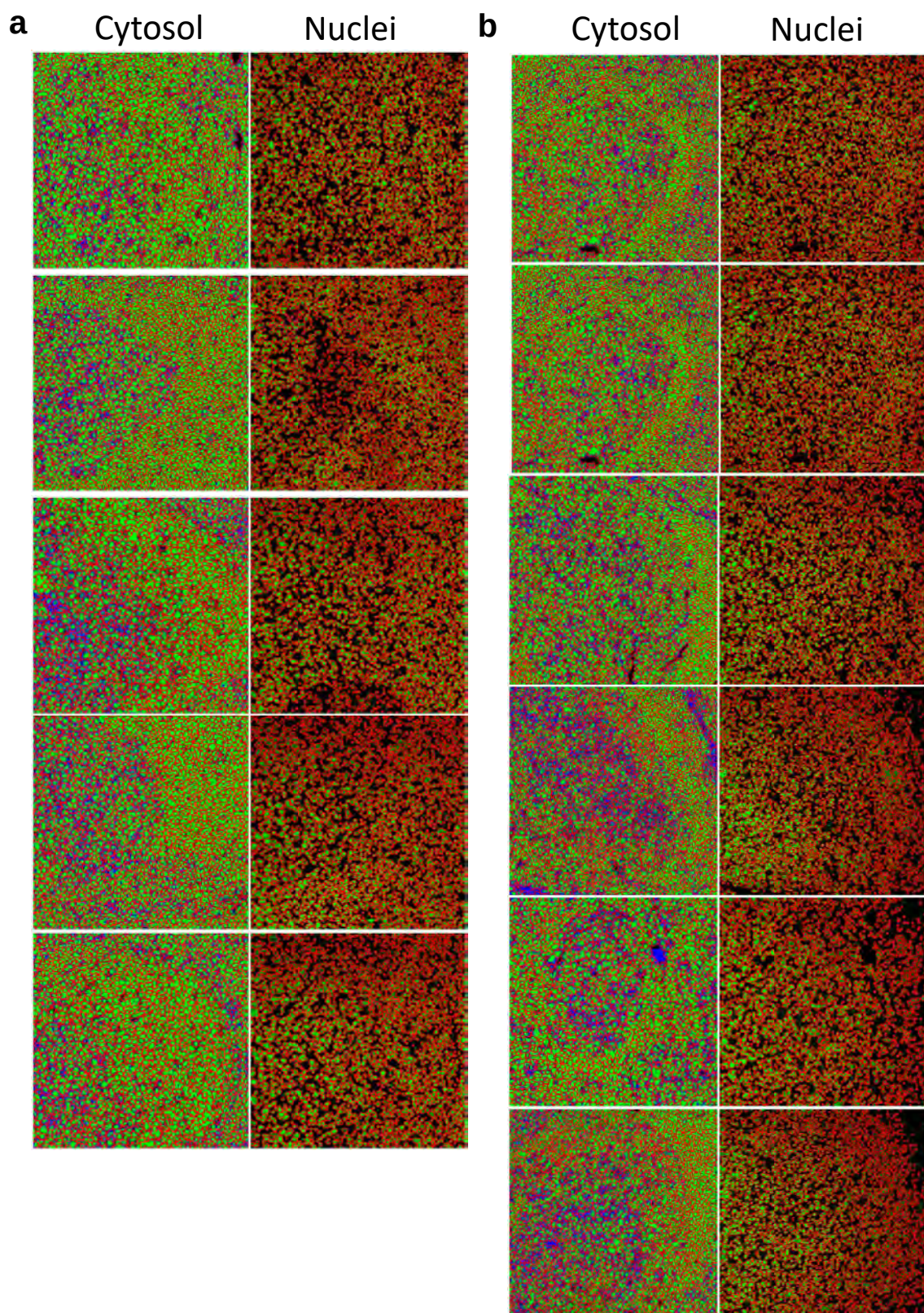
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Supplementary Fig. 51. Comparison of single-cell segmentation in tonsil tissue. The image of cytosolic (blue) and nuclei (green) channels is shown by combining multiplex IMC markers **a** with the corresponding cytosol segmentation **b**, nuclei segmentation **c**, and SIMS image segmentation **d**. **e** Comparison of the average segmented cell areas for cell cytosol and nuclei region as well as nuclei region using the SIMS modality (n = 1530 cells). Mann-Whitney-Wilcoxon test was two-sided with Bonferroni correction (ns: $0.05 < p$, ****: $p \leq 0.0001$). All box plots with center lines showing the medians, boxes indicating the interquartile range, and whiskers indicating a maximum of 1.5 times the interquartile range beyond the box.

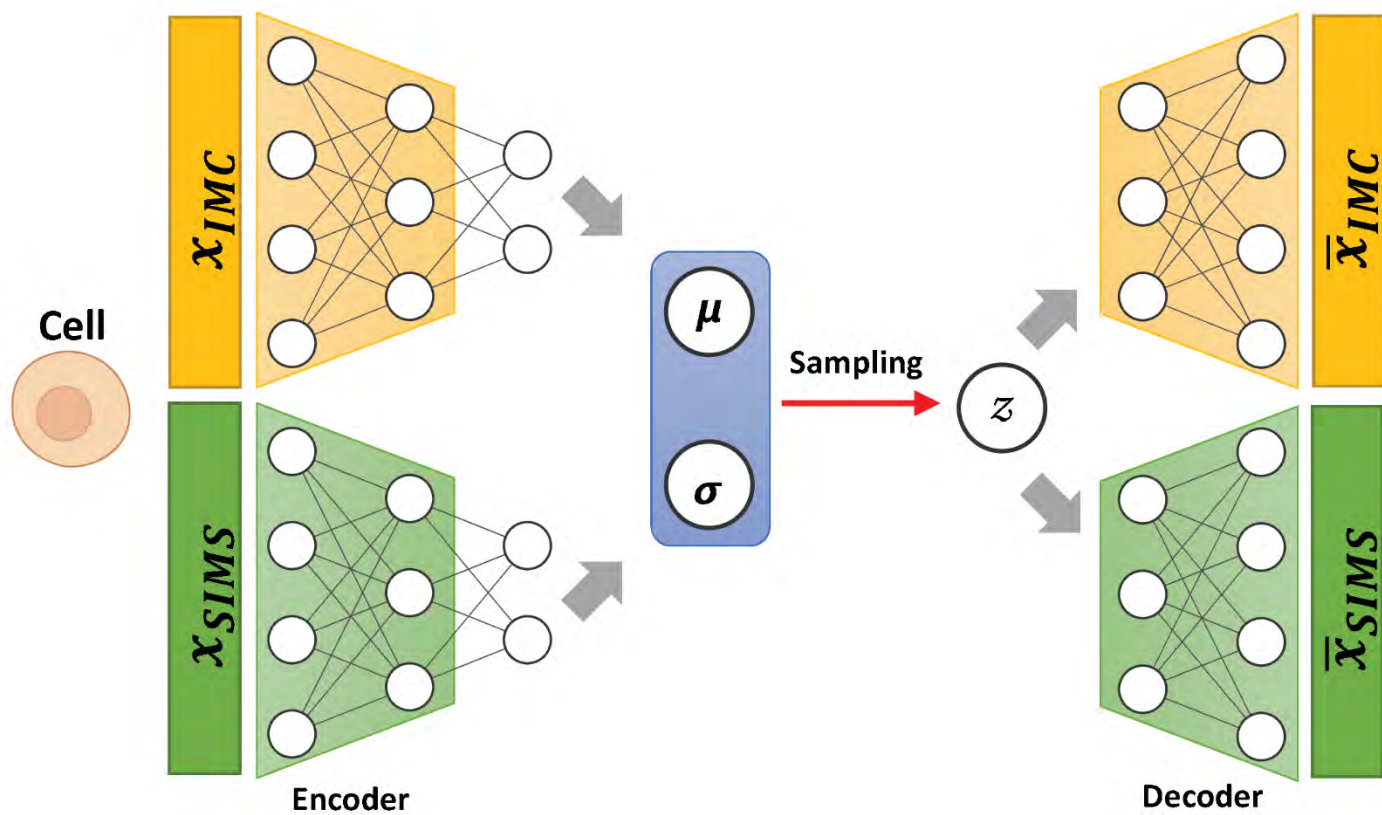


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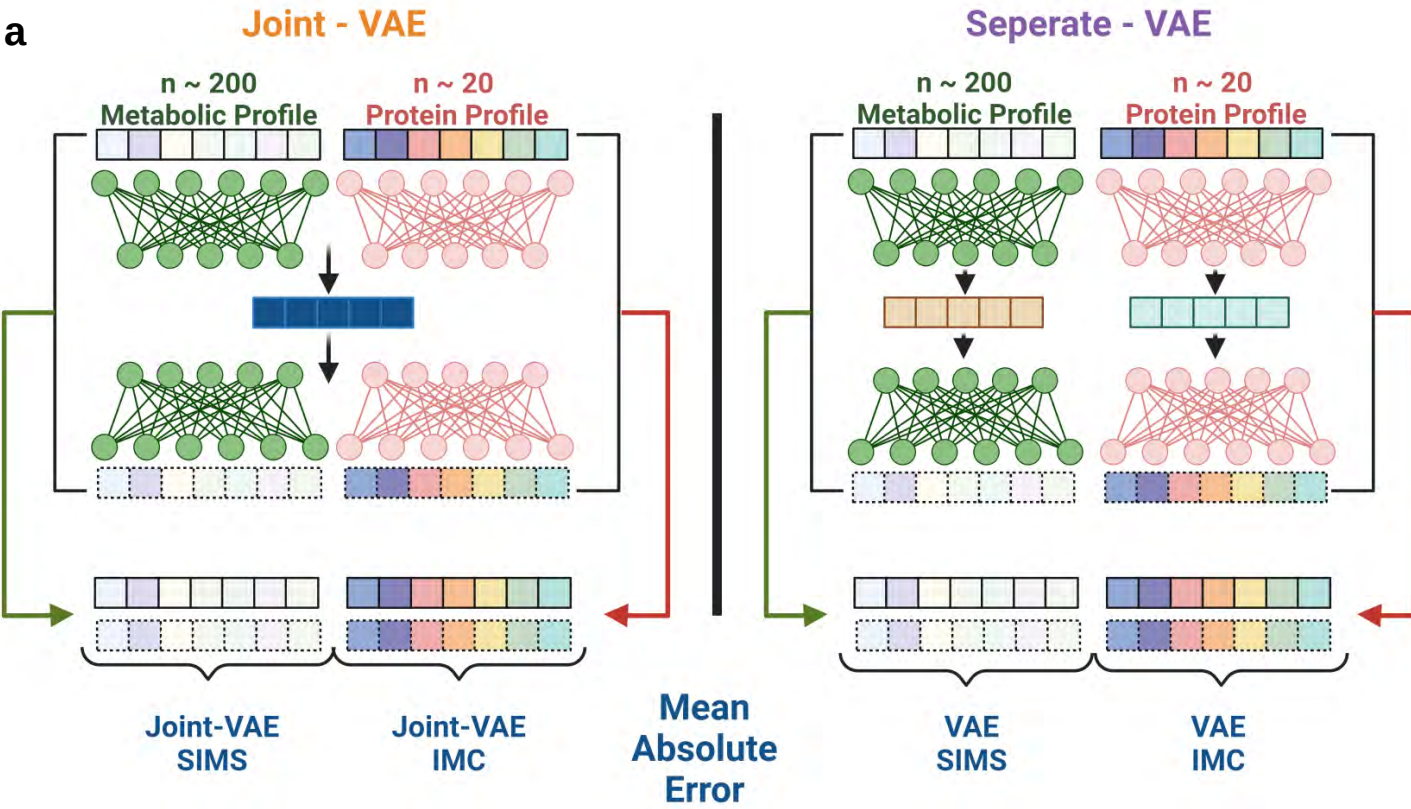
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394 **Supplementary Fig. 52.** Comparison of single-cell segmentation in tonsil tissue donor A **a** and donor E **b** with
395 *DeepCell* algorithm using cytosolic markers (left) and corresponding segmentation from nuclei channels in
396 SIMS image (right).

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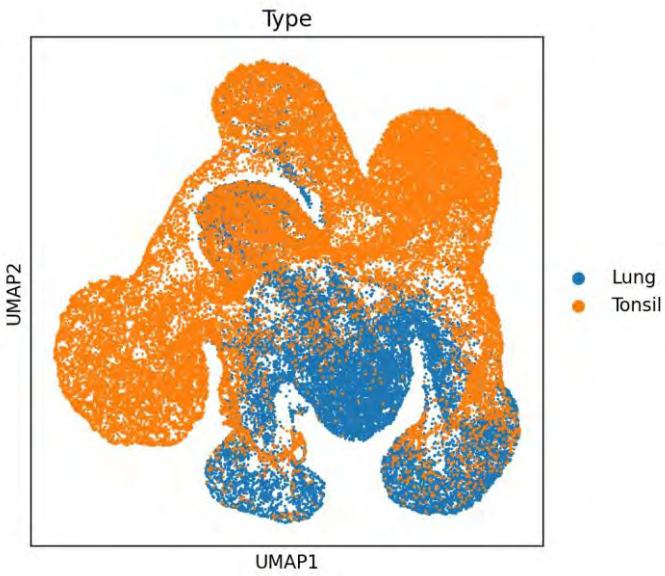
400 **Supplementary Fig. 53.** VAE architecture for metabolite protein joint embedding. The input of the network is
401 the metabolite and protein profiles and the output is the reconstructed profiles.
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b

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Dataset	Model	Modality	MAE
Tonsil	Joint-VAE	SIMS	0.6103
		IMC	0.0701
	VAE	SIMS only	0.6381
		IMC only	0.0813
Lung	Joint-VAE	SIMS	0.5074
		IMC	0.0822
	VAE	SIMS only	0.5589
		IMC only	0.0802
Endometrium	Joint-VAE	SIMS	0.6201
		IMC	0.1093
	VAE	SIMS only	0.6307
		IMC only	0.1019



Supplementary Fig. 54. VAE embeddings for joint metabolite and protein profiles

- a** Schematic representation of joint embedding reconstruction compared to individual modality reconstruction using VAE for metabolite and protein profiles. Created with Biorender.com
- b** Table showing the mean absolute error of reconstruction from joint-VAE and single modality VAE across the imaged datasets.
- c** UMAP shows the VAE joint embedding using common protein markers in lung cancer and tonsil datasets.

412 **Supplementary Tables**

Method	Capability	Mass imaging	Resolution	Cell type specificity	Cell target region	Cell size comptability
scSpaMet	Integrates metabolite and specific protein labeling at single-cell level	TOF-SIMS/IMC	500-nm resolution	25 Cell types	Cytosol and nucleus	Small cell size
3D-SMF	Detect tissue region metabolites	TOF-SIMS	500-nm resolution	20 protein markers	Tissue region	None
SpaceM	Integrates MALDI with confocal imaging	MALDI	10-µm resolution	2 Cell types	Whole-cell	Large cell size
SEAM	Only detect nuclear metabolites	TOF-SIMS	1-µm resolution	No true cell types	Nucleus	Small cell size
Raman Spectrometry	Only detects limited metabolic targets	Microscopy	300-nm resolution	No true cell types	Cytosol and nucleus	Small cell size

413 **Supplementary Table 1. Comparison of emerging single-cell spatial metabolomics technologies**

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Method	Integration modality	Require matching markers	Same cell measurements	Same tissue
MARIO	Protein and transcriptome measurements. (CODEX / CITE-seq)	Partially shared features/markers	No	No
MNNs, Scanorama, Conos	The same modality designed for scRNA-seq batch effect removal	Total matching	No	No
Seurat v4	Protein, ATAC, and RNA	Partial matching cell type	Yes	Yes
Liger	DNA methylation, chromatin accessibility, and RNA	Partial matching cell type	No	No
SpaceM	No integration	No matching. Define cell type using a light microscope	Yes	Yes
SEAM	Metabolites and Transcriptome	No matching. Analysis of correlative expression from sequential tissues	No	No

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Supplementary Table 2. Comparison of emerging single-cell spatial multi-omic analysis

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Marker	Clone	Metal Tag	Dilution
CD20	H1	161Dy	1:400
CD3	Polyclonal	170Er	1:100
CD4	ERP6855	156Gd	1:200
CD45RO	UCHL1	173Yb	1:50
CD68	KP1	159Tb	1:50
CD8a	C8/144B	162Dy	1:100
FoxP3	236A/E7	155Gd	1:30
Pan-Keratin	C11	148Nd	1:100
Granzyme B	EPR20129-17	167Er	1:100
Ki-67	B56	168Er	1:50
SMA	1A4	141Pr	1:200
Collagen type I	Polyclonal	169Tm	1:300
E-cadherin	24E10	158Gd	1:50
Histone 3	D1H2	171Yb	1:50
Vimentin	D21H3	143Nd	1:100
CD11b	EMP1344	149Sm	1:50
CD44	IM7	153Eu	1:100
CD31	EPR3094	151Eu	1:100
CD45RA	HI100	166Er	1:50
CD11c	Polyclonal	154Sm	1:100
Intercalator	—	191Ir/193Ir	1:400

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Supplementary Table 3. List of antibodies, their conjugated metal tags, their clones, and their concentrations used for imaging mass cytometry in lung cancer microarrays.

Core number	Sex	Pathology Diagnosis	Grade	Stage	Type
B5, F7	M	Adenocarcino ma	3	IIIA	Malignant
C6, F4	M	Adenocarcino ma	3	IIA	Malignant
D4, E4, E6	M	Adenocarcino ma	3	IB	Malignant

Supplementary Table 4. BS04081a patient specifications for cancer type, stage, and grade.

Donor No.	Sex	Pathology Diagnosis
A-E	M	Normal Tonsil Tissue

Supplementary Table 5. HuFPT161 patient specifications for tonsil tissue.

Marker	Clone	Metal Tag	Dilution
CD3	Polyclonal	170Er	1:100
CD45RO		166Er	1:50
CD45RA	UCHL1	173Yb	1:50
CD8a	C8/144B	162Dy	1:100
Pan-Keratin	C11	148Nd	1:100
SMA	1A4	141Pr	1:200
E-Cadherin	24E10	158Gd	1:50
Vimentin	D21H3	143Nd	1:100
Intercaltor		192/193Ir	1:100

Supplementary Table 6. List of antibodies, their conjugated metal tags, their clones, and their concentrations used for imaging mass cytometry in endometrium tissues

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